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PHYSIOLOGY

JACQUES LOEB
EDITOR

VOLUME II

BERKELEY
THE UNIVERSITY PRESS
1904-1905

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PHYSIOLOGY

Vol. 2, No. 1, pp. 1-3. November 2, 1904

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THE CONTROL OF HELIOTROPIC REACTIONS IN FRESH WATER CRUSTACEANS BY CHEMICALS, ESPECIALLY CO_2.

(A Preliminary Communication.)

BY

JACQUES LOEB.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

In the discussion of the causes of animal heliotropism in my former papers I pointed out that in all probability the light produced chemical changes in the eye or skin of the animals, and that these changes were responsible for the heliotropic reactions. It seems to me that if this were true it should be possible to control the heliotropic phenomena very easily by chemical means. This has thus far not been possible. In recent experiments on the toxic effects of acids and alkalis I made incidentally an observation, which led to the result that in fresh water crustaceans the heliotropic reactions can indeed be controlled beautifully by specific chemical substances. In this preliminary communication only a brief summary of the results thus far obtained will be given.

1. Gammarus pulex is naturally negatively heliotropic. This reaction can easily be demonstrated by transferring the animals to a dish with fresh or distilled water or by stirring them up. Usually their positive stereotropism makes them cling to solid bodies in the water or to the walls of the dish, and in this case they pay little or no attention to the light.

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2. If CO_2 is allowed to bubble through the water, or if the animals are thrown into water previously charged with CO_2, they become at once outspokenly positively heliotropic. Other acids, *e.g.*, hydrochloric, or oxalic or acetic, act in the same way. A concentration of about m/500 is sufficient for this effect. Boracic acid, however, has no such effect, even in as strong solution as m/10.

3. Similar results can be obtained by certain narcotics, such as ether, chloroform, paraldelhyd, alcohol, or esters.. If the animals are thrown into a 2½m solution of ethyl alcohol they become at once positively heliotropic, but very soon they become semi-paralyzed and drop to the bottom of the dish. In this semi-paralyzed stage their motions are no longer coordinated and they cease to react to the light in an outspoken way. Ether is effective in a m/6 solution. Chloroform, which is very slightly soluble, acts in a considerably weaker concentration, but its narcotic effects are so powerful that the heliotropic effects last only a few seconds. The action of esters is the most striking of all the representatives of this group of bodies. Ethylacetate makes Gammarus instantly positively heliotropic at a concentration of m/50. But in the case of the esters it is not impossible that the action of the free fatty acid contributes to the result.

4. Among the salts, only one has been found whose effect can be compared with that of CO_2 or the other acids, namely, NH_4 Cl, or rather all the NH_4 salts, NH_4HO included. A m/25 solution of NH_4Cl makes the Gammarus all suddenly positively heliotropic.

5. Alkalis, with the exception of NH_4HO, do not make them positively heliotropic; they only excite them. Urea and the salts (with the exception of NH_4 Cl and to a small degree possibly K salts) excite them, but leave them either negatively heliotropic or cause only a scattering of the animals. Pure oxygen or hydrogen does not make them positively heliotropic, but leaves them markedly negatively heliotropic.

6. Cyclops can be made energetically positively heliotropic by CO_2 or any of the other acids effective in Gammarus. Positively heliotropic Cyclops are made negatively heliotropic by NaHO. 1½cc n/10 NaHO added to 50cc of distilled water

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suffice to make strongly positive Cyclops negatively heliotropic. The young Cyclops require less alkali than the adult Cyclops.

7. I have not yet made many experiments with Daphnia, and can thus far only state that they can be rendered very outspokenly positively heliotropic by CO_2 or any other acid or by ethylacetate.

8. Attempts to make *seawater* Gammarus positively heliotropic by CO_2 have failed. This is not due to properties of the seawater, for fresh-water Gammarus when put into seawater (in which they remain negatively heliotropic) can be promptly made positively heliotropic by CO_2.

9. The fact that such substances as are produced in the animal body itself, *e.g.*, CO_2 or other acids or ammonia, influence the reactions of the animals to light, may explain a number of peculiarities in the heliotropic behavior which we have thus far not been able to understand. The fact that during the daytime the algae at the surface of lakes consume the CO_2, may contribute toward making these animals negatively heliotropic and thus forcing them to migrate downward.

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PHYSIOLOGY

Vol. 2, No. 2, pp. 5-30. December 9, 1904

FURTHER EXPERIMENTS ON HETEROGENEOUS HYBRIDIZATION IN ECHINODERMS.[1]

(With two figures in the text.)

BY

JACQUES LOEB.

I.—Introduction.

While the successful hybridization experiments in Echinoderms which have been formerly published refer only to different species in the same family, I have, I believe, given last year for the first time unassailable proof that by combining the sexual cells of individuals of different families of Echinoderms, living larvae can be produced.[2] Although I was only able at that time to report the successful crossing between sea-urchins *(Strongylo centrotus)*♀ and one species of starfish *(Asterias ochracea)*♂, I have since convinced myself that the eggs of the sea-urchin mentioned can be successfully fertilized with the sperm of several, perhaps all, species of starfish, and especially with the sperm of a representative of another family of Echinoderms, namely the *Ophiuridae*. I believe also that I have succeeded in fertilizing the eggs of sea-urchins with the sperm of individuals of a third family, namely, the *Holothurians*. Since, however, I have not been able to complete these experiments with the sperm of *Holothurians* to my entire satisfaction, their discussion must be omitted in this publication.

[1]Translated from *Pflüger's Archiv.*, 1904, Bd. 104, S. 325, by John Bruce MacCallum.

[2]LOEB, *University of California Publications*, Physiology, Vol. I, pp. 1, 39, and 85. *Pflüger's Archiv.*, Bd. 99, S. 323 and 637.

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In order to designate briefly the hybridization of the individuals of different families, the term *heterogenous* hybridization will be applied. The crossing of various species of the same family may be spoken of as *homogenous* hybridization. For the fertilization of the eggs of one species with the sperm of the same species the term pure fertilization may be used.

It is well known that even homogenous hybridization is not always very successful. The possibility of heterogenous hybridization has commonly been justly doubted. If we consider the grounds of the difficulty we find, as far as I know, that only two causes have been mentioned, namely, in the first place, the mechanical difficulties which stand in the way of the entrance of a spermatozoon into the egg of a species belonging to a different family; and secondly, the fact that the body fluids of one animal are in general poisonous for the cells of an unrelated species. The latter possibility would be particularly important in heterogenous hybridization, and has indeed been given by v. Dungern as the cause of the failure of the sea-urchin sperm to fertilize the egg of the starfish. With regard to the mechanical difficulty, the excessive size or the unsuitable form of the spermatozoon has been suggested, and this, in the case of eggs which possess a micropyle, is quite plausible. I believe, however, that the experiments, formerly published by me, show that a heterogenous hybridization which is impossible or only exceptional under normal conditions, succeeds on an enormous scale when a slight change is made in the chemical constitution of the surrounding fluid, namely, in its reaction. While in normal sea-water the fertilization of the sea-urchin egg by the sperm of *Asterias* is only exceptional and occurs only in isolated eggs, the the process is successful in van't Hoff's solution which has been made alkaline. This is true also, as I have found this year, for sea-water which has been rendered alkaline, and is true for the fertilization of the sea-urchin egg not only by the sperm of *Asterias,* but of any starfish and of the *Ophiuridae* thus far tried.

II.—Heterogeneous Hybridization in Alkaline Sea-water.

It was desirable for various reasons to replace the artificial solutions used in my former experiments by natural sea-water.

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If one desires to make the sea-water whose reaction is normally neutral, alkaline or acid, it must be remembered that the sea-water possesses in the bicarbonates and diphosphates, regulators which tend to keep its reaction neutral. If we add a small amount of NaHO to the sea-water, part of the alkali is neutralized, and the concentration of the hydroxyl ions in the sea-water is smaller than corresponds to the quantity of the NaOH added. Dr. Cottrell has undertaken at my suggestion to determine by means of the gas battery the concentration of free hydrogen ions in sea-water to which various quantities of alkali or acid have been added; and the results of this investigation, which is of general importance in the physiology of the body fluids are to be published subsequently.

Now, with regard to the methods of experiment in hybridization, it is to be noted that only such sea-water was used as was first freed from all living spermatozoa by being heated to 60°. This measure of precaution is *absolutely necessary,* and after my experiences I must insist that hybridization experiments in which this precaution is not taken are to be considered in general as of no value. Care must also be taken in the sterilization of the instruments and hands of the experimentor, and especially of the surface of the animal. It is absolutely necessary to clean the animals with a strong stream of fresh water. This is the more necessary if, as is often the case, the male discharges the sperm spontaneously into the water. I have made it a rule in these cases not to make use of such material for experiment. In addition to the precautionary measures against infection of the cultures by the sperm of the same species as that from which the eggs are obtained, still other precautions are necessary to provide against the possibility of a parthenogenetic development of the eggs. This source of error is guarded against by control-experiments, as I have already shown and shall discuss again later on. We shall now pass on to a description of the experiments.

To each 100 c.c. of sterilized sea-water were added respectively 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 c.c. n/10 NaHO. Four to six drops of sea-urchin's eggs *(Strongylocentrotus purpuratus* is the only form of sea-urchin which is considered in

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this work) were placed in each solution, and then the sperm was added. In general the eggs and the sperm from more than one animal were used.

To the eggs of *Strongylocentrotus,* which were present in the solutions, the sperm of *Asterias capitata* was added. Each solution received exactly the same quantity of sperm. After ten minutes a number of the eggs in the sea-water which contained more than 1.0 c.c. n/10 NaHO per 100 c.c. sea-water, formed the typical fertilization membrane. After two hours the fertilized eggs showed on counting the result which is set down in Table I.

TABLE I.

Fertilization of the eggs of *Strongylocentrotus* by the sperm of *Asterias capitata.*

Nature of Solution	Percentage of fertilized eggs after 2 hours.
100 c.c. normal sea-water	0%
100 " " " + 0.25 c.c. n/10 NaHO	0
100 " " " + 0.5 " " "	0
100 " " " + 0.75 " " "	0
100 " " " + 1.0 " " "	ca 20
100 " " " + 1.25 " " "	over 50
100 " " " + 1.50 " " "	ca 10
100 " " " + 1.75 " " "	ca 5
100 " " " + 2.0 " " "	ca 5

The following day the following facts were observed: Those eggs which had been fertilized were in the blastula stage. In normal sea-water I found no fertilized eggs. In 100 c.c. sea-water + 0.25 c.c. NaHO I found one fertilized egg; in 0.5 NaHO about 2% of the eggs were now fertilized, but they were in very early stages of segmentation, many in the 2-4- cell stage, a proof that the fertilization took place in this case very late. In 100 c.c. sea-water + 0.75 NaHO the majority of the eggs were fertilized, but only about 2% had reached the blastula stage. In the solutions with more NaHO a development of the eggs fertilized in the first two hours had taken place, but the number of the fertilized eggs had not increased. Many repetitions of this experiment with the sperm of *Asterias capitata* gave almost the same result.

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When the sperm of the twenty-rayed starfish *(Pycnopodia spuria)* was used the number of the fertilized eggs was considerably smaller. Here also the fertilization occurred in the first ten minutes only in sea-water with a high degree of alkalinity. Table II describes such an experiment.

TABLE II.

Fertilization of the eggs of *Strongylocentrotus* with the sperm of *Pycnopodia spuria.*

Nature of Solution.	Percentage of fertilized eggs 2 hours and 20 minutes after the fertilization.
100 c.c. normal sea-water	0%
100 " " " + 0.25 c.c. n/10 NaOH	0
100 " " " + 0.5 " " "	0
100 " " " + 0.75 " " "	0
100 " " " + 1.0 " " "	0.8
100 " " " + 1.25 " " "	2.0
100 " " " + 1.5 " " "	2.0
100 " " " + 1.75 " " "	0.5
100 " " " + 2.0 " " "	0

On the next morning there were found in the normal sea-water and in the solutions with 0.25 and 0.5 NaHO no fertilized eggs. The eggs which were fertilized on the first day had developed. I was of the opinion at first that perhaps the sperm of *Pycnopodia* was not so ripe as that of *Asterias,* and that this explained the relatively small number of fertilizations. But throughout six weeks I repeated this experiment at various times, with always the same results. In most cases I used also the sperm of different males. From this it must be supposed that the sperm of *Pycnopodia* does not unite with the eggs of *Strongylocentrotus* or fertilize it so easily as does the sperm of *Asterias capitata.* Experiments with the sperm of another group of starfish, namely, *Asterina,* seem to support this supposition. I obtained the sperm of *Asterina* in a condition of greatest maturity. Nevertheless the number of *Strongylocentrotus* eggs which were fertilized by the sperm of *Asterina* was still less than in the case where the sperm of *Pycnopodia* was employed. In the most favorable cases about 1% of the eggs were fertilized. The fertilization took place here most abundantly in 100 c.c. sea-water with 1.0 to 1.5 c.c. n/10 NaHO. The fertilized eggs developed. I

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thought that the degree of relationship perhaps plays a rôle. This, however, seems to be contradicted by the fact that the fertilization of the *Strongylocentrotus* eggs by the sperm of a brittle star takes place almost as easily as that with the sperm of *Asterias,* although indeed *Asterina* and *Asterias* are more nearly related morphologically than *Asterina* and the brittle stars, which even belong to different families. The brittle star with which we are concerned here may be obtained at Pacific Grove, where the experiments were carried on, only at very low tide. In consequence of this these experiments were made earlier than the others already published, and they are therefore not entirely comparable with them, because in the experiments with the brittle star I added Na_2CO_3, instead of NaHO. I found that often as many as 20- 50% of the eggs of *Strongylocentrotus* were fertilized by the sperm of the brittle star, and that the optimal concentration of the alkali which must be added to the sea-water for this hybridization is lower than in the experiments with the starfish sperm.

In my hybridization experiments published last year, only the sperm of *Asterias ochracea* was employed. Since I worked then almost solely with artificial solutions, I repeated this year these experiments with sea-water rendered alkaline. By the addition of 1.25 c.c. n/10 NaHO to 100 c.c. sea-water as many as 50% and more of the eggs of *Strongylocentrotus* could be fertilized by the sperm of *Ocracea* in less than ten minutes. With the addition of 0.5 or still less NaHO to 100 c.c. sea-water, usually no fertilization of the *Strongylocentrotus* eggs occurred during the first 24 hours. The same is true of normal sea-water which was obtained from the ocean, although in such cases I observed last year occasionally some fertilizations. We may therefore regard it as a general result that if 1-2 c.c. n/10 NaHO be added to 100 c.c. of normal sea-water, the eggs of the sea urchin *(Strongylocentrotus)* will be fertilized by the sperm of many, perhaps all, starfish in about 10-30 minutes. This percentage of eggs which may be so fertilized is relatively high for the sperm of *Asterias ochracea* and *capitata,* and is much lower for the sperm of twenty-ray starfish *(Pycnopodia spuria),* and is very low for *Asterina.* On the contrary, the percentage of fertilized eggs

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and larvae is great in the fertilization of the same eggs with the sperm of a brittle star. It is possible, though not probable, that the difference is due only to the different condition of maturity of the sperm.

III.—Hybridization in Neutral and Acid Sea-water.

Last year I had, as above mentioned, repeatedly observed that eggs of *Strongylocentrotus* could be fertilized by the sperm of *Asterias ochracea* in exceptional cases also in normal sea-water which was obtained from the ocean at Pacific Grove. The number of eggs fertilized in this way was nevertheless small, about one egg in 10,000. It struck me also that the fertilization in normal sea-water took place relatively late, usually many hours after the addition of the sperm; while the fertilization of the same eggs by the same sperm occurred in less than half an hour in alkaline van't Hoff's solution. When I found this year in repeating these experiments that in sea-water rendered alkaline the fertilization of the eggs of *Strongylocentrotus* by the sperm of *Asterias ochracea* occurred in about ten minutes, while in neutral sea-water no fertilization of the same eggs by the same sperm took place in the first 24 hours, I extended the experiment over a greater length of time and found that 30-48 hours after the addition of the sperm individual eggs were actually fertilized and developed. The number of the eggs fertilized in this way was extremely small, and it was necessary to seek for a long time before a fertilized egg could be found. The determination of the percentage is very difficult; I found perhaps one or two fertilized eggs in a watch glass full of eggs.

These observations suggest the idea that the hydroxyl ions exert an accelerating action on the beginning of fertilization. Systematic experiments were therefore made with sea-water whose alkalinity was increased by the addition of various quantities of Na_2CO_3. With the suitable degree of alkalinity the eggs were fertilized in less than one-half hour. If a little less Na_2CO_3 were added about an hour was required before the fertilization appeared. With the addition of still less Na_2CO_3 the fertilization occurred only after many hours. Exact time measurements are not possible in these cases, since in these weak or

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almost subliminal solutions the few eggs that are fertilized are not fertilized simultaneously. It is only possible to determine the point at which fertilized eggs are first found in greatest abundance; and such an estimation shows that with a certain optimal concentration of hydroxyl ions (corresponding to the addition of about 1.5 c.c. n/10 NaHO to 100 c.c. sea-water) the eggs of *Strongylocentrotus* are fertilized by the sperm of *Asterias ochracea* in about ten minutes, that with diminishing concentration of the hydroxyl ions the time which elapses before the appearance of the first fertilization increases, although the increase is not a regular one.

I then sought to find out whether a hybridization of the eggs of *purpuratus* by the sperm of *Asterias ochracea* is possible also in sea-water to which a small quantity of HCl was added. With the addition of 0.1, 0.2, 0.3 to 0.5 c.c. n/10 HCl to 100 c.c. sea-water I found after 48 hours occasionally an egg with a fertilization membrane which was in the first stages of segmentation and subsequently developed regularly.

The same experiments were repeated with the sperm of the other starfish. The general result was that if one waited a long enough time—generally 48 hours—a fertilization of the sea-urchin's eggs by the sperm of starfish occurred in normal sea water. The number of eggs which are fertilized under these conditions is always very small, and never exceeds about one egg in 10,000. The experiments with the sperm of the brittle star gave an interesting corroboration of this statement. The optimal degree of alkalinity at which the eggs of *Strongylocentrotus* are fertilized immediately and in large number by the sperm of brittle stars is smaller than for the sperm of the starfish. Accordingly also the fertilization of these eggs in normal sea-water by the sperm of the brittle stars (if it indeed occurs) takes place eight to sixteen hours after the addition of the sperm, and often in greater number than on the addition of the sperm of the starfish.

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IV.—Hybridization in Sea-water to Which Various Other Salts Have Been Added.

It is possible to produce immediate fertilization of the eggs of the sea-urchin by the sperm of the starfish by adding to the sea-water an appropriate quantity of Na_2CO_3. Under these conditions the number of the fertilized eggs is as great as with the addition of the equivalent quantity of NaHO. If to 100 c.c. of sea-water about 0.3 c.c. ⅝m Na_2CO_3 be added, a fertilization of the sea-urchin's eggs by the sperm of *Asterias ochracea* occurs in less than one hour; and if the optimal quantity of Na_2CO_3 be employed the fertilization results in about ten minutes. The number of the fertilized eggs also is about as great as in the case of the addition of NaHO (30-50%). In the fertilization of the eggs of *Strongylocentrotus purpuratus* by the sperm of *Asterias capitata, Pycnopodia spuria* and *Asterina,* the optimal addition of Na_2CO_3 is about 0.3 c.c. of a ⅝m solution of this substance to 100 c.c. sea-water. In the case of brittle stars a considerably smaller quantity suffices, *e.g.,* 0.1 c.c. of this solution or even less. If 0.25 to almost 2.0 c.c. of a ⅜m NaH CO_3 solution be added to 100 c.c. sea-water, usually everything goes on as in normal sea-water. If, however, a larger quantity of the bicarbonate be added, a very large number of eggs are fertilized after 12 to 24 hours. I observed in one case where the sperm of *Asterias ochracea* and the eggs of *Strongylocentrotus* were used, that in 100 c.c. sea-water + 3.0 c.c. ⅜m NaH CO_3 the majority of all the eggs were fertilized 24 hours after the addition of the sperm, and that on the next day about 20% of all the eggs reached the blastula stage. In the mixture of 100 c.c. sea-water + 2 c.c. ⅜m NaH CO_3 only ½% were fertilized. With the addition of 4 c.c. ⅜m NaH CO_3 to 100 c.c. sea-water fertilization took place in the course of the first 24 hours in the majority of the eggs; they, however, did not develop. I presumed that in these solutions carbonic acid escaped and a part of the bicarbonate was converted into carbonate, on account of which the solution assumed an alkaline reaction. Tests made with phenolphthalein, in fact, showed that all solutions which contained 3 c.c. ⅜m

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NaH CO_3 or more in 100 c.c. sea-water had a distinct alkaline reaction after 24 hours. Corresponding with this the fertilization occurred also in shallow vessels somewhat sooner than in deep vessels. In these experiments the accelerating influence of the NaH CO_3 was due to its partial transformation into Na_2CO_3.

Experiments with the addition of 0.5 to 3.0 c.c. m/2 sodium tartrate solution and neutral potassium citrate solution had no distinct influence on the hybridization. Addition of sulphates to the sea-water had no accelerating effect on the hybridization. We may say that with our present knowledge the concentration of the hydroxyl ions in the sea-water is the decisive variable which determines the rapidity of the fertilization of the sea-urchin's egg by the sperm of the starfish, and the sperm of the brittle stars, and also determines the relative number of the fertilized eggs.

V.—On the Changes Which the Spermatozoa of the Starfish Undergo in Alkaline Sea-water.

The observation that in normal (neutral) sea-water isolated sea-urchin's eggs may be fertilized after a very long time (24 hours or more), while in alkaline sea-water or in certain artificial solutions which are made alkaline the fertilization of a large percentage of the eggs takes place very rapidly, suggests that either the spermatozoon of the starfish or the egg of the sea-urchin, or both, must suffer a change before heterogenous fertilization is possible, and that this change ocurs more rapidly and to a greater extent when the sea-water has a certain degree of alkalinity than when it is neutral.

In order to arrive at a more detailed elucidation of this, the following experiments were made. Sperm of *Ochracea* was placed in 100 c.c. sea-water + 2.0 n/10 NaHO. At the same time eggs of *Strongylocentrotus* were placed in a solution of the same kind, but in another vessel. Immediately after the beginning of the experiment a drop of eggs and a drop of sperm were mixed in a watch glass. Six minutes elapsed before the first egg formed a fertilization membrane. Then, however, the membrane formation took place rapidly in a large number of eggs.

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At a second test eggs and sperm were mixed in a watch glass eight minutes after having been placed in the alkaline sea-water. In this instance only three minutes elapsed before the first egg showed fertilzation by the formation of the membrane, and immediately after this many eggs formed their fertilization membranes.

Thirteen minutes after placing the eggs and sperm in the alkaline sea-water, a drop of eggs and a drop of sperm were, as a third experiment, taken from the alkaline sea-water and mixed. No eggs were fertilized. Similar results were obtained with all later tests.

It was possible to show that only the sperm was made powerless to carry out the fertilization, while the eggs had suffered no diminution in their capacity for fertilization. For if fresh sperm of *Ochracea* be added to these eggs in the alkaline sea-water (100 c.c. and 2 c.c. m/10 NaOH) the fertilization takes place. If, however, the sperm which has been 13 minutes in the alkaline sea-water be added to fresh eggs of *Strongylocentrotus* in the alkaline solution, no fertilization occurs.

It may also be directly observed that in the sperm a modification has occurred. It shows, namely, a marked agglutination or clumping. In the beginning of this agglutination the individual spermatozoa were still motile, and the motility of the spermatozoa hastened the formation of greater aggregates of single spermatozoa. Later, however, their motility ceased. In the transition step of the clumping and of the loss of motility, the power of the spermatozoa to fertilize is lost.

These facts explain also the observation which I made that in 100 c.c. sea-water + 2.0 NaHO a fertilization of the sea-urchin's eggs by the sperm of the starfish occurred only in the first ten minutes; and that in waiting longer, the number of eggs which were fertilized in this solution did not increase. I repeated these experiments, but instead of a solution of 100 c.c sea-water + 2.0 n/10 NaHO, a solution of 100 c.c. sea-water + 1.2 n/10 NaHO was used. Immediately after the sexual cells had been placed in this solution (but in separate vessels) a drop of eggs and sperm were mixed in a watch glass After eight minutes the first fertilization took place, and the number of fer-

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tilized eggs increased rapidly. After 11 minutes a second fertilization was undertaken. This time only 2½ minutes elapsed before the fertilization began. The experiments were repeated after 16, 21, and 27 minutes, and always only about two to three minutes elapsed before the fertilization began. Then, however, the agglutination of the spermatozoa began, and the time which was necessary for the fertilization increased rapidly, and the number of eggs which were fertilized diminished rapidly. While in the first and second experiments about 50% of the eggs were fertilized, in the third and fourth about 30%, in the fifth only 5%, and in the sixth 1% were fertilized, and then the fertilization soon ceased.

After an hour and twenty minutes the sperm of *Ochracea* had lost completely its power of fertilization. The eggs, on the contrary, could still be fertilized after remaining twenty-four hours in the alkaline sea-water if they were mixed with normal sperm of the same or of another male *Ochracea.*

There is no doubt that the loss of the power of fertilization in alkaline sea-water is due to a change in the spermatozoon and not in the egg, and that this loss is associated with the appearance of the agglutination of the spermatozoa. My colleague Taylor, to whom I showed this agglutination, informed me that the agglutination of the starfish spermatozoa in alkaline sea-water bears a marked similarity to bacterial agglutination such as occurs in Vidal's typhoid fever test.

Whether the same changes, however, which finally result in the agglutination of the spermatozoa of *Ochracea* form at an earlier stage a preparatory condition for the possibility of their fertilizing the eggs of the sea-urchin is conceivable, but not yet demonstrable. That it is possible follows from the fact that about eight minutes elapse before the sea-urchin's eggs are fertilized by starfish sperm in sea-water at the optimal alkalinity, while very soon, under the influence of this alkali this latent period diminishes to three minutes, and remains at this point until the power of fertilization is lost. In the first minutes the alkali must produce changes in the spermatozoon of the starfish which make the fertilization of sea-urchin eggs possible, or shorten the time of impregnation, or both. If the sperm of *Aste-*

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rias ochracea and the eggs of *Strongylocentrotus* be left separately in alkaline sea-water for some time, and are then mixed in a greater quantity of normal sea-water, no fertilization of the eggs usually takes place.

I have convinced myself that the agglutination in alkaline sea-water is to be observed not only with the sperm of *Asterias ochracea,* but also with the sperm of *Asterias capitata, Pygnopodia spuria,* and *Asterina.* In the experiments with the sperm of *Ophiuridae* I have omitted to investigate this point.

The sperm of the sea-urchin, on the contrary, behaves quite differently.

VI.—On the Influence of the Reaction of the Sea-water on the Pure Fertilization of the Eggs of the Sea-urchin.

My experiments published last year have already shown that the solutions in which the sea-urchin's eggs are fertilized in the greatest numbers by the sperm of *Asterias,* hinder or render impossible the fertilization of the same eggs by the sperm of the same species; provided that the sperm be washed for some time (about five minutes) in this solution before it is brought into contact with the egg. While the alkaline van't Hoff's solution produces a change in the sperm of the starfish by which it becomes more suitable for the fertilization of the sea-urchin's egg, this same solution produces a contrary action in the sperm of the sea-urchin.

I undertook this year experiments on the fertilization of the sea-urchin's eggs by sperm of the same species in alkaline and acid sea-water. Eggs and sperm of *Strongylocentrotus* were first placed separately for five minutes in the solution mentioned above (before they were mixed). In one experiment there was added 100 c.c. sea-water, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 c.c. n/10 NaHO. Ten minutes after the mixture of the eggs and sperm almost all the eggs were fertilized in the normal sea-water and in the sea-water with 0.25 c.c. NaHO. In sea-water with 0.5 c.c. NaHO about half of the eggs were fertilized; in sea-water with 0.75 to 1.25 NaHO an occasional fertilized egg was found, while in sea-water with 1.5 to 2.0 NaHO no eggs were fertilized. The eggs remain thus unfertilized by the sperm of

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their own species in this alkaline sea-water in which they are most easily and in greatest number fertilized by the sperm of the starfish.

Experiments were now carried on by the same methods in acidified sea-water. To each 100 c.c. sea-water were added 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 c.c. n/10 HCl. In the sea-water which contained 0.1 to 0.7 c.c. HCl almost all the eggs were fertilized; in the sea-water with 0.8 to 1.0 cc. m/10 HCl, on the contrary, only a few were fertilized. As a control the eggs of the same female were fertilized in the same solution by the sperm of *Asterias ochracea.* In sea-water with 1.0 to 2.0 NaHO about 30 to 50% of the eggs were fertilized in about 20 minutes; in sea-water with less NaHO or with acid, not a single egg was fertilized in this time.

If the eggs of the sea-urchin be brought into contact with the sperm of the sea-urchin in alkaline sea-water without being previously washed, the eggs became fertilized. I inquired therefore how soon in alkaline sea-water the sea-urchin sperm loses its activity in producing fertilization, and whether an agglutination of the sperm occurs with the beginning of its sterility.[1] Eggs of *Strongylocentrotus* and sperm of the same form were separately placed in 100 c.c. sea-water to which 1.0 c.c. n/10 NaHO was added. Immediately a couple of drops of the eggs and the sperm were mixed in a watch glass. In about a minute the fertilization began, and in 1½ minutes 30 to 40% of the eggs were fertilized. Four minutes after the beginning of the experiment a fresh quantity of eggs and sperm from the alkaline sea-water was combined in a watch glass, and in this case also the fertilization began in one minute; the number of the fertilized eggs, however, increased much more slowly than in the first test.

Ten minutes after placing the eggs and the sperm in the alkaline sea-water a quantity of the eggs and of sperm was combined again in a watch glass. After three minutes one fertilized egg was found, but no more eggs were fertilized. Later mixtures

[1] I beg the reader to observe that here the agglutination of the spermatozoon to one another, and not to the egg, is meant. The latter is an entirely different phenomenon, which apparently depends on other conditions. The agglutination, as the term is used here, corresponds to the phenomenon of clumping or precipitation of suspended particles, although the aggultinated sperm does not necessarily fall to the bottom.

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of the sperm and the eggs which had remained in the alkaline sea-water gave uniformly negative results.

That this depends again only on the injury to the sperm and not to the eggs may be shown by the fact that if to the eggs there be added normal sperm of the same male, a fertilization of all the eggs takes place in the alkaline sea-water as well as in normal sea-water.

Now with regard to the nature of the injury to the sperm bodies, an agglutination of the sperm is not responsible. The *sea-urchin's spermatozoa differ from the spermatozoa of the starfish in that the former do not agglutinate in alkaline sea-water* even if 3.0 c.c. n/10 NaHO be added to 100 c.c. sea-water. The injury consists rather, it seems, in a loss of motility on the part of the spermatozoa of sea-urchins in sea-water which has been rendered alkaline (and in alkaline van't Hoff's solution). This lack of motility may be again done away with by bringing the spermatozoa back into neutral sea-water, provided that they do not remain too long in the alkaline sea-water. Spermatozoa of *Strongylocentrotus* which were no longer in a condition to fertilze the eggs of *Strongylocentrotus* after remaining ten minutes in 100 c.c. sea-water + 1.0 c.c. n/10 NaHO, were placed in normal sea-water for ten minutes after they ceased to fertilize the eggs. Eggs were then added. About ten minutes elapsed before the sperm was in a condition to fertilize the eggs, and the number of eggs which were fertilized was then only ½%. Plainly only a small number of spermatozoa regained their power of motility.

If sea-water be used to which more than 1.0 c.c. n/10 NaHO has been added to each 100 c.c. the capacity for fertilization disappears with considerably greater rapidity. But, as already mentioned, there never occurs an agglutination of sperm (that is, sticking of the spermatozoa to one another). I desired now to determine whether acids do not perhaps act in the same way on the spermatozoa of the sea-urchin as alkalis do on the spermatozoa of starfish. I employed therefore solutions of 100 c.c. sea-water with 1.0, 1.5, and 2.0 c.c., respectively, of n/10 HCl. If the sea-urchin's spermatozoa are placed in a solution of 100 c.c. sea-water + 2.0 c.c. n/10 HCl, they are not motile at once, but

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begin to become motile only after several minutes. Not until perhaps 20 minutes have elapsed does fertilization of a small number of sea-urchin's eggs take place in this solution. With less acid the motility begins rapidly and more eggs are fertilized, and indeed the fertilization occurs earlier. A clumping of the sea-urchin's sperm frequently takes place in acidified sea-water, but not always.

I am therefore not in a position to state with certainty that the clumping (agglutination) of the spermatozoa of the starfish with one another in alkaline sea-water forms a later stage in the series of changes which make the fertilization of the sea-urchin egg by starfish sperm possible. If this were correct, there would follow perhaps a more general criterion for the possibility of heterogenous hybridization.

In the close relation which exists between the process of the formation of aggregates (agglutination) by particles suspended in fluid, and their electrical charge, it is necessary to inquire whether perhaps the electrical charges of the spermatozoa of starfish and sea-urchins show differences when in contact with the eggs of sea-urchins which run parallel with the power of these spermatozoa to fertilize the eggs of the sea-urchin in alkaline, neutral, or acid solutions. Experiments concerning this are in progress in this laboratory.

VII.—On the Influence of the Quantity of the Sperm on the Fertilization.

In my earlier work I have mentioned that I added more sperm when the eggs of the sea-urchin were to be fertilized by the sperm of starfish, than when the sperm of the sea-urchin was used. The reason was that in general more sea-urchin's eggs were fertilized at higher concentration of the starfish sperm. If a hybridization in neutral or too weak alkaline solution is being dealt with, a long time must elapse before any eggs are fertilized. We believe that these observations may be explained by supposing that in this latest period a change takes place in the spermatozoon which is necessary for its power to fertilize the egg. But this change occurs in neutral sea-water only in an extremely small percentage of the spermatozoa. It is evident that the

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number of eggs which are fertilized in such a sub-alkaline solution must increase with the concentration of the sperm. On the other hand we have seen that in solutions with too high a concentration of free hydroxyl ions, the spermatozoa very soon suffer so that the percentage of these which remain available for the fertilization becomes very small. Also within certain limits an increase in the concentration of the sperm increases the chances for the fertilization of the eggs.

It further follows from what has been said, that, if the extremes mentioned be avoided, *i.e.*, if one chooses alkaline solutions, of not too small and not excessive concentration of hydroxyl ions, the eggs of the sea-urchin might be fertilized by the sperm of the starfish even with relatively small concentration of sperm. I diluted the sperm of *Asterias ochracea* with sea-water to such a degree that the suspension in an ordinary dropping pipette began to appear turbid. Eggs of *Strongylocentrotus* were then placed in a series of vessels, of which each contained 100 c.c. sea-water + 1.2 c.c. n/10 NaHO. Each vessel contained four drops of eggs. To the various vessels were added a different number of drops of the suspension of sperm mentioned, namely, 3, 6, 12, 24, 48, and 96 drops. Table III gives the results of this experiment.

TABLE III.

Quantity of sperm added	Number of fertilized eggs After 10 minutes	After 30 minutes
3 drops	40%	40%
6 "	50	70
12 "	70	70
24 "	80	90
48 "	80	80
96 "	80	80

If it is remembered that the addition of three drops of sperm in the dilution mentioned, to 100 c.c. sea-water is about the requisite concentration in which the eggs of the *Strongylocentrotus* are fertilized by the sperm of the same species, it is evident that in a solution suitable also for heterogenous hybridization the concentration of the sperm need not be much greater than in the pure fertilization.

Schücking made incidentally the assertion that by grinding together sperm and eggs the fertilization of *Asterias* eggs by the

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sperm of Arbacia occurs successfully; and that by grinding together *Strongylocentrotus* eggs with the sperm of *Asterias* larvae were formed some of which reached the gastrula stage.[1] Since, however, control experiments necessary for his assertion are absolutely wanting, it is difficult to perceive what caused him to regard the formation of swimming larvae in these cases as due to the grinding of the sperm with the eggs. In his experiments on the fertilization of the eggs of starfish by the sperm of sea-urchins everything points to the fact that in this case parthenogenetic development occurred, as we shall see in the next section. With regard to the fertilization of the sea-urchin's eggs by the sperm of *Asterias,* we have seen that such a fertilization takes place occasionally and on a small scale in normal sea-water, without a grinding together of the eggs and sperm being necessary. I considered it therefore desirable to submit Schücking's statement to a test. Sperm of *Asterias ochracea* was added in varying concentrations to eggs of *Strongylocentrotus* in normal sea-water. Eggs of the same culture and spermatozoa of the same male *Asterias* were ground in a mortar. The energy and the duration of the grinding and the concentration of the sperm varied. In some tests almost pure sperm, with only a trace of sea-water, was employed. In no case did I find either in the grinding experiments or in the control tests a single fertilized or developing egg in the first 24 hours. The experiment was often repeated with various cultures, and the same negative results obtained. Since Schücking has excluded neither the possibility of an infection by sea-urchin's sperm, nor that the sea-urchin's eggs would have developed under the influence of *Asteria* sperm without grinding, I cannot at present agree with his statement that the grinding of the sperm and eggs accelerates their fertilization. The fusion of the spermatozoon and egg is caused by the movement of the spermatozoa and possibly by surface tension when the spermatozoon comes in contact with the protoplasm of the egg. I had thought of the possibility that when the sum of the surface tension between egg and spermatozoon, and water and spermatozoon becomes smaller than the

[1]SCHÜCKING, *Pflüger's Archiv.* Bd. 97, S. 58, 1903.

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tension between sea-water and egg protoplasm the fusion of the egg and sperm must take place. At present, however, every proof for the correctness of this hypothesis is still absent.

VIII.—On the Behavior of the Sea-urchin's Eggs in Alkaline Sea-water When no Sperm is Added.

If we assert that a sea-urchin's egg is fertilized by the sperm of a starfish, it rests on the observation of the fact that upon the addition of living starfish spermatozoa the sea-urchin's eggs form the membrane characteristic for fertilization and then develope; while the same eggs without the addition of living sperm show neither of the two phenomena. The possible sources of error are, as I have already shown in part in earlier publications, infection of the eggs with sperm of the same species, artificial membrane formation due to changes in the chemical nature of the solution and artificial parthenogenesis of the sea-urchin's eggs. Only control experiments serve to eliminate these sources of error. As a control against the infection with sperm of the same species, eggs of the same culture which remained in sea-water without the addition of sperm were used. Only where these control eggs remained absolutely unfertilized was an experiment regarded as admissable. With regard to the elimination of the two other sources of error, there was prepared almost constantly for each experiment a series of control experiments in which the eggs were placed, without sperm, in the same solutions as those used for the hybridization. In the alkaline van't Hoff's solutions used last year a membrane formation and development of the eggs never took place without the addition of sperm. With the alkaline sea-water, on the contrary, it was somewhat different. Sometimes, but very seldom, eggs were found which formed a membrane in alkaline sea-water. These eggs did not develope.

In order to produce such a membrane formation by alkalis much more alkali must be added than is necessary for the hybridization. But a membrane formation is very rare, and plainly depends on individual peculiarities of the eggs of certain female sea-urchins. It is to be understood that the eggs which behave in such a way were excluded from this series of experiments.

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With regard now to artificial parthenogenesis, there occurs in unfertilized eggs which are placed in sea-water to which 1 c.c. or more of n/10 NaHO has been added to every 100 c.c. of sea-water, a beginning of a parthenogenetic segmentation after many (about 12 to 24) hours—a fact which I have already published in my first detailed work on artificial parthenogenesis.[1] The more NaHO is added to the sea-water, the greater is the percentage of eggs which show the parthenogenetic segmentation, and the sooner this occurs. But the segmentation in these cases never leads to the development of a blastula; the egg never goes past a two or a (mostly irregular) six or eight celled stage. The egg then gradually dies, ordinarily by breaking down into individual segumentation cells. It is to be observed also that this kind of parthenogenetic segmentation cannot lead to any deception with regard to the fertilization of the sea-urchin's eggs by the sperm of starfish in alkaline sea-water, since in the latter case the fertilization membrane is immediately formed and the segmentation proceeds as it does in normally fertilized eggs. It is, however, necessary that all experiments on hybridization in artificial solutions should be accompanied by control experiments in which the eggs of the same female which were used for the experiment remain in the same solutions without sperm.

As in the experiments in artificial parthenogenesis, one will also in the experiments on heterogenous hybridization, obtain the best results if he employs the most minute and painstaking precautions and control experiments.

IX.—On the Fertilization of the Eggs of Starfish by the Sperm of the Same Species, and by the Sperm of Sea-urchins.

Since the unfertilized eggs of starfish can be caused to develope into larvae by means of internal changes in the egg (perhaps the formation of carbonic acid?) without any external interference, these eggs are not very well suited for experiments on hybridization. One could experiment successfully then with the eggs of starfish if it were possible in any way to recognize in each case whether they develope parthenogenetically or on account of the entrance of a spermatozoon, as is the case in the

[1] LOEB, *American Journal of Physiology*, Vol. 3, p. 434, 1900.

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eggs of sea-urchins where the formation of the fertilization membrane serves as a criterion for fertilization. I have recently observed that the eggs of *Asterina* when they become fertilized form indeed a fertilization membrane which is as distinct if not more distinct than that formed in fertilized sea-urchins' eggs, while this membrane is wanting in eggs of *Asterina* which develope parthogenetically. These eggs may be used to determine whether the solutions in which the eggs of starfish are best fertilized by the sperm of starfish are identical with those in which the same sperm best fertilizes the eggs of the sea-urchin. I found that the eggs of *Asterina* (which first remained, for the sake of ripening, three hours in normal sea-water without the addition of sperm) were fertilized immediately and in large numbers in normal sea-water by sperm of the same species. Since the same spermatozoa are in a position to fertilize the eggs of the sea-urchin in normal sea-water either not all or only exceptionally and then after many hours, this observation shows that a different property of the spermatozoa of starfish is necessary for the fertilization of the sea-urchin's eggs than for the eggs of the starfish.[1]

In sea-water which was made alkaline the percentage of the fertilized eggs of *Asterina* after the addition of sperm of the same species was not greater than in normal sea-water. If, however, more than 1.25 c.c. n/10 NaHO were added to 100 c.c. sea-water the number of fertilized eggs decreased. Likewise the addition of acid to the eggs diminished the number of fertilized eggs. I cannot regard these experiments as conclusive, since their number is too small. I intend to carry on further researches in this direction.

With regard now to the fertilization of the eggs of *Asterina* by the sperm of the sea-urchin *(Strongylocentrotus purpuratus)*,

[1] I have repeatedly pointed out the analogy between fertilization and infection. I wish to make a further addition to the series of these analogies. When it is not possible to infect an animal species with the tubercle bacillus of man, it is usually decided that the tubercle bacillus of man and that of the animal form used are not identical. If this is the only reason for denying the identity of the two microörganisms, the above experiments show that a slight chemical difference in the tissue fluids of the two animal forms may be responsible for the circmustance that the human tubercle bacillus does not prosper so well in the animal form referred to. The correctness of this idea might be tested by experiments analogous to the hybridization experiments.

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I have in no case so far succeeded with certainty. In all control experiments where the eggs remained without the addition of sperm the number of eggs developing was just as great as in the corresponding solutions to which the sea-urchin's sperm had been added. The eggs of *Asterina* which developed on the addition of sea-urchin's sperm were always without a fertilization membrane. It is, however, to be remembered that the number of the sea urchin's eggs which were fertilized by the sperm of *Asterina* was smaller than the number of the sea-urchin's eggs which were fertilized by the sperm of any one other species of starfish. It is perhaps possible that the germinal substance of *Asterina* and *Strongylocentrotus* are especially incompatible. While no observation which is free from objection, of the fertilization of the starfish egg by the sperm of the sea-urchin exists, it is still possible that an extension of the experiments may lead to positive results. In this, however, it must not be overlooked that the eggs of the starfish, as opposed to the eggs of the sea-urchin, can develope in normal sea-water into normal larvae without any external intervention; and that, further, on these grounds, all experiments of this sort on the eggs of the starfish are accompanied by a high degree of uncertainty; if one does not work with forms whose eggs (as in *Asterina)* indicate the fertilization by the typical membrane formation. It is also necessary in this case to make sure that an appearance of this sort does not occur in the unfertilized eggs.

X.—On the Specific Capacity for Development and Duration of Life of the Various Hybrids between the Sea-urchin and the Starfish.

As is well known, Landois has shown that if into the veins of an animal the blood of a different species be injected, there occurs a destruction of the red-blood corpuscles; and that these unfavorable symptoms are absent only when the species whose blood is used for the interchange are very closely related. Friedenthal has used this result to decide the question which animals may be regarded as related by blood. He came to the conclusion that a blood relationship exists between the ass and the horse,

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the hare and the rabbit, man and the anthropoid apes. He showed further that these facts are perhaps connected with the problem of hybridization. "It is certainly not an accident that it is known that, with most of the animals which show identical kinds of blood, a fertile crossing of the species takes place. Ass and horse, hare and rabbit, dog and wolf bring into the world living hybrids. If a crossing of species between rat and mouse, house cat and ozelot has thus far been unsuccessful, on account of the difference in size of the animals, it would be worth while to determine by means of artificial fertilization, whether the possibility of the production of living hybrids does not coincide with the results of the blood reactions in such a way that only those species are capable of a successful hybridization the blood of which is not reciprocally hemolytic."[1]

As far as I know, no researches exist on the question whether the blood cells of the sea-urchin are destroyed in the blood of the starfish or the brittle star, and *vice versa*. In the zoölogical system, however, the sea-urchin, starfish and brittle star belong to three different families, and a successful crossing there should be impossible. It is nevertheless surprising that a crossing of the representatives of these families is still successful in so far that developing bastard larvae may be produced between them.

If, however, the development of these heterogenous bastard larvae be compared with those of the pure breeding, striking differences appear in the power of living of the heterogenous hybrid and the pure sea-urchin larvae. These differences become evident usually not until the second or third day, and consist in an enormously greater tendency to die on the part of the heterogenous hybrids.

If the first stages in segmentation of the heterogenous bastard larvae (*Strongylocentrotus* eggs and *Asterias* sperm) be watched, the segmentation occurs in the beginning in almost the same way morphologically as in eggs fertilized by the sperm of the same species. The essential difference is perhaps that in the latter case all the eggs of the same culture are usually found to be in the same stage of segmentation; while with heterogenous hybrids

[1]FRIEDENTHAL, H., *Archiv für Physiologie Jahrg.* 1900, S. 404.

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there usually exist great differences, in that all eggs are not fertilized at the same time and perhaps also do not develope with equal rapidity.

After twenty-four hours the difference between the heterogenous and purely fertilized eggs is much more conspicuous. The larvae of the latter usually swim about at this time, while the former are found only in transition to the blastula stage. Further, many, perhaps the majority, of the heterogenously fertilized eggs are already dead or have remained in very early stages in the segmentation. The purely fertilized *Strongylocentrotus* eggs, on the contrary, are as a rule all alive and all in the same larval stage. After two days the pure *Strongylocentrotus* larvae proceed usually to the pluteus stage, while the heterogenous larvae, even in the most favorable cases, are found only in the transition stage to the gastrula. The latter larvae also usually swim at the bottom, while the *Strongylocentrotus* larvae of pure breeding swim in large numbers at the surface or rise from the bottom. After three days the difference in the cultures is quite surprising. The heterogenous bastards are almost all dead, the pure larvae almost all alive.

In my efforts to breed the heterogenous hybrids, their sudden death in large numbers between the second and third days was always especially striking and annoying. One gains the impression that the cultures of these larvaes have been suddenly poisoned, and perhaps a slow poisonous action is exerted by certain substances of the starfish or brittle star sperm upon the eggs of *Strongylocentrotus.* Still a small number of these larvae remain alive and develop slowly to an early pluteus stage. Early pluteus stages of this sort are found not until the fourth or fifth day in the heterogenous culture, while in the pure culture this stage is reached usually after forty-eight hours (at the same temperature). Figs. 1 and 2 are camera drawings of two such heterogenous bastards four days old *(Strongylocentrotus* egg and sperm of *Asterias ochracea).* The intestine is in an early stage of development and the skeleton exists only in the *Anlage.* The other details are not shown, since Professor Heath of Stanford University, to whom I am indebted for the drawings, intends to carry out a morphological analysis of the heterogenous plutei

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produced in such experiments. I find among the numerous sketches which I have made myself a few heterogenous plutei that are better developed than those represented in these drawings.

FIG. 1.

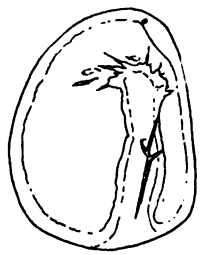

FIG. 2.

Now are these heterogenous plutei capable of a growth into a further stage of development? This naturally can be determined only by breeding experiments, which will doubtless be laborious and difficult. When one considers that up to the present time only a few investigators have succeeded in the raising of even pure larvae of the sea-urchin beyond the pluteus stage.

So far the statements concern only hybrids between the eggs of *Strongylocentrotus* and the sperm of *Asterias ochracea* and *capitata*. With the sperm of the other starfish and brittle stars I have so far bred no plutei. The bastards between sea-urchin and brittle star seem to show a very great tendency to die early.

Since the fertilizations in these cases were made in almost all experiments in artificial alkaline solutions or alkaline sea-water, it seemed possible that the high mortality of the heterogenous bastards depended not so much on the foreign sperm as on the abnormal reaction of the solution or the sea-water. I convinced myself that the latter condition has no part, or only a small part, in the excessive mortality of the heterogenous bastards. If the eggs immediately or one or two hours after the fertilization by starfish sperm, were replaced in normal sea-water the mortality was just as great as when they remained in 100 c.c. sea-water + 1.0 n/10 NaHO. On the other hand, the mortality of the sea-urchin's eggs which were fertilized by sperm of the same species was not markedly greater in a solution of 100 c.c. sea-water + 10 n/10 NaHO than in normal sea-water. The greater

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mortality of the heterogenous bastards is dependent on the heterogeneous character, or rather, some physico-chemical incompatibility of the sexual products.

In conclusion, I wish to express my thanks to my colleagues of Stanford University, in whose laboratory at Pacific Grove these experiments were carried out.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 3, pp. 31-42. December 9, 1904.

THE INFLUENCE OF CALCIUM AND BARIUM ON THE SECRETORY ACTIVITY OF THE KIDNEY.[1]

(Second Communication.)

BY

JOHN BRUCE MACCALLUM.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

In previous publications[2] it was shown that subcutaneous or intravenous injections of small quantities of solutions of certain salts, including the saline purgatives, produce not only increased peristalsis, but also an increased secretion of fluid into the intestine. This was found to be true also when the solutions were applied locally to the peritoneal surfaces of the intestine. It was suggested that the main actions of saline purgatives consist in the production of increased peristaltic movements, and of increased secretion of fluid into the intestine; and that the semi-fluid foeces which are produced by saline purgatives are the result not of decreased power of absorption by the intestine, but of an increased secretion of fluid into the intestine. It was further shown that the administration of calcium or magnesium chloride tends to suppress the peristaltic movements and the secretory activity of the intestine. Attention was specially called to the marked action of barium chloride in the production of violent peristaltic movements and ringlike constrictions in the intes-

[1] Reprinted from the *Journal of Experimental Zoölogy*, Vol. I, No. 1, May, 1904. A preliminary report of these experiments was published in the University of California Publications, *Physiology*, January 15, 1904, Vol. I., No. 10. p. 81.

[2] J. B. MACCALLUM: *American Journal of Physiology*, Vol. X, No. 3, p. 101, 1903, and Vol. X, No. 5, p. 259, 1904.

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tine, and also in the production of an increased flow of fluid into the intestine. It was also pointed out that the production of these activities in the intestine by the purgative salts, and their suppression by calcium and magnesium is analogous to the production and suppression of rhythmical contractions in voluntary muscles described by Loeb.[1] The antagonism which has been shown by Loeb to exist between the actions of many sodium salts on the one hand, and calcium and magnesium salts on the other was further illustrated by these experiments.

It seemed possible then in the light of these facts that the activities of the kidney might be controlled in the same way as those of the intestine. Since it is well known that many sodium salts have a distinct diuretic action, it seemed conceivable that calcium or magnesium might act as an antidiuretic. In order to decide this point I have made a series of experiments in which I have found that the relation of many of the salts to the activity of the kidneys is similar to that which they bear to the glandular activity of the intestine.

METHODS.

The following experiments were carried out mainly on rabbits; a few dogs also were used. In all cases morphine was given as an anaesthetic. The rabbits received 3-5 cc. 1 per cent. solution of morphine hydrochlorate subcutaneously; the dogs in addition to this dose of morphine were given ether when necessary.

The urine was collected by catheterising the ureters or by tying a cannula in the bladder. The latter method was employed in all cases except those in which it was necessary to observe the difference between the amounts secreted by the two kidneys. In placing a cannula in the bladder a small incision was made in the abdominal wall. The bladder, which usually contains a considerable quantity of urine, was then lifted out of the body cavity, and the abdominal wall sewed up around the neck of the bladder, so that the intestines could not be forced out. A purse-string suture was then made in the fundus of the bladder and an incision made in the bladder wall within the suture. In

[1] J. LOEB: *Festschrift für Fick*, 1899; *Archiv für die gesammte Physiologie.* 1902, XCI, p. 248.

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this way the urine could be removed, and the cannula securely tied in. Care was taken to allow no urine to collect in the bladder, so that the measurements given in the tables represent all the urine that was secreted during each period. The simple catheterisation of the bladder through the urethra may be easily performed in rabbits, but this method is unsatisfactory when it is necessary to obtain the exact amount of urine secreted in a given time, since it is impossible to tell whether the bladder is at any time entirely empty.

Solutions of the salts whose actions were tested were introduced into the body intravenously. In rabbits a hypodermic needle was placed in a vein of the ear; in dogs the fluid was forced into one of the superficial veins of the lower limb. When small quantities were injected a hypodermic syringe was used; when larger amounts were introduced a pressure apparatus was employed. This was the apparatus commonly used in injection work, consisting of a pressure bottle connected on one side with a water tap, and on the other with a graduated bottle containing the solution to be injected. In this way a constant pressure could be obtained, and the quantity of fluid injected in a unit of time accurately controlled. By causing the fluid to pass through a coil of rubber tubing immersed in hot water before reaching the needle, the solution could be kept constantly at the body temperature. Some of the details of the apparatus were suggested to me by an apparatus used by Dr. M. H. Fischer in this laboratory. For such infusions into the blood only m/8 solutions were employed; in subcutaneous injections stronger solutions were used. Except in those cases where it was necessary to obtain the effect of the salt on the normal flow of urine, the secretion was considerably raised and kept constant by the uniform infusion of m/8 NaCl solution throughout the experiment. The effect of the other salts was then obtained by allowing small quantities of m/8 solutions to flow into the vein along with the NaCl solution. In other instances these salts were injected into a vein of one ear while the NaCl solution was at the same time flowing into the opposite ear. In these experiments the ear of the rabbit was securely tacked to the board, and the needle kept from slipping out of the vein by means of bull-dog forceps.

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EXPERIMENTS.

The results of the experiments on the actions of calcium and barium may be best seen in the following tables:

1. Dog—small terrier—cannula placed in right ureter.

Urine secreted in 1st 10 minutes	3.6 cc.
2d 10 minutes	3.6 cc.
8 cc. m/8 $CaCl_2$ injected into vein of leg.	
Urine secreted in 1st 10 minutes	2.4 cc.
2d 10 minutes	2.2 cc.
3d 10 minutes	1.8 cc.
4th 10 minutes	1.6 cc.
5th 10 minutes	1.4 cc.
10 cc. m/2 sodium citrate injected subcutaneously.	
Urine secreted in 1st 10 minutes	1.6 cc.
2d 10 minutes	2.3 cc.
3d 10 minutes	3.1 cc.
4th 10 minutes	3.6 cc.

In this case the secretion of urine gradually decreased after the injection of calcium chloride until the amount collected in a unit of time was less than half of the initial amount. The addition of sodium citrate to the blood counteracted this effect so that the rate of secretion again approached the normal. These effects are more striking when the quantity of urine secreted is increased by the introduction of normal salt solution into the blood, as shown in the following experiment:

2. Rabbit—cannula placed in bladder. No urine flowed in the first or second periods of 10 minutes before the NaCl solution was injected.

Time.	Salts other than NaCl injected.	m/8 NaCl injected in cc.	Urine in cc.
10.10	.....	10	....
10.15	.....	10	....
10.20	.....	5	0.5
10.40	.....	10	0.8
11.00	.....	10	0.5
11.20	.....	5	1.0
11.40	.....	10	2.8
12.00	.....	10	6.0
12.00	5 cc. m/8 $CaCl_2$ intravenously		
12.05	5 cc. 5m/8 $CaCl_2$ subcutaneously.		
12.20	.....	5	0.2
12.40	.....	10	1.8
1.00	.....	10	0.8
1.00	5 cc. m/8 sodium citrate intravenously.		
1.20	.....	10	2.2
1.40	.....	5	3.6

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In this experiment, although the flow of urine has been considerably increased by the injection of m/8 NaCl, the introduction of $CaCl_2$ markedly suppresses the secretion. The flow of urine remains small for an hour, although a somewhat greater quantity of fluid is forced into the blood than in the previous hour. This suppression of urine is at once counteracted by the injection of sodium citrate.

The following table (3), which represents only the latter half of an experiment, shows roughly the duration of the action of smaller doses of calcium.

3. Rabbit—cannula in bladder—injections intravenous.

Time.	Salts other than NaCl injected.	m/8 NaCl injected in cc.	Urine in cc.
9.25	.....	....	....
11.40	.....	150	64.5
11:45	.....	10	6.6
11.50	.....	10	5.6
11.55	.....	10	6.2
12.00	.....	10	7.4
12.05	.....	10	9.5
12.05	5 cc. m/8 $CaCl_2$		
12.10	.....	5	2.2
12.15	.....	10	0.8
12.20	.....	10	1.2
12.25	.....	10	1.6
12.30	.....	10	2.8
12.35	.....	8	3.0
12.40	.....	5	4.5
12.45	.....	0	4.8
12.50	.....	0	5.1
12.55	.....	0	6.2

As shown here and in other experiments, the action of calcium is only temporary. I have found also that magnesium chloride in many cases has an antidiuretic action similar to that of calcium chloride. The suppression of urine, however, is not so marked as with calcium.

As shown in the following experiment (4) barium chloride in very small doses has a strong diuretic action. Although it is much more powerful in this respect than sodium citrate, the increased flow of urine which it causes may be suppressed by the injection of calcium chloride.

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4. Rabbit; injections intravenous.

Time.	Salts other than NaCl injected.	m/8 NaCl injected in cc.	Urine in cc.
10.20	.....	....	....
10.30	.....	20	....
10.40	.....	10	....
10.50	.....	20	1.2
11.00	.....	32	2.8
11.10	.....	28	5.8
11.20	.....	20	6.1
11.30	.....	10	8.2
11.40	.....	10	8.3
11.40	⅛ cc. m/8 $BaCl_2$		
11.50	.....	10	14.4
12.00	.....	10	18.0
12.10	.....	10	12.4
12.10	⅛ cc. m/8 $BaCl_2$		
12.20	.....	10	18.4
12.30	.....	10	16.4
12.30	5 cc. m/8 $CaCl_2$		
12.40	.....	10	8.6
12.50	.....	10	4.0
1.00	.....	10	2.0
1.10	.....	10	2.4
1.20	.....	5	3.4
1.50	¾ cc. m/8 $BaCl_2$		
1.30	.....	8	6.4
1.40	.....	10	8.2
1.50	.....	10	8.6
1.20	¼ cc. m/8 $BaCl_2$		
1.55	.....	10	1.8
2.00	.....	10	0.6
2.10	.....	10	0.0
2.20	.....	....	0.0
2.30	.....	....	0.0

In the uniform injection of considerable quantities of normal salt solution into the blood, the flow of urine, after about an hour, becomes fairly constant. If an average amount of 1 cc. in 1 minute be introduced, the secretion of urine during the first two or three hours is usually slightly less than the amount of fluid injected. After this time, when no other salts have been added, the quantity injected and the quantity secreted may become approximately equal. As shown in experiment 4, however, the addition of a minute quantity of $BaCl_2$ (less than ½ cc. m/8 solution) to the blood causes the flow of urine to increase markedly,

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so that the quantity secreted in a unit of time is far in excess of the quantity of fluid introduced into the blood. If, however, while this active secretion is going on, 5 cc. m/8 $CaCl_2$ solution be injected into the blood, the flow of urine rapidly decreases, although the total quantity of fluid added to the blood remains constant. The further addition of $BaCl_2$ again increases the secretory activity, so that the quantity secreted in 10 minute periods which has fallen from 16.4 to 2, under the influence of $CaCl_2$ is again raised to 8.6 by the injection of the barium salt. An apparently contradictory thing, however, happens when a larger amount of barium chloride is suddenly added to the blood. As shown in the foregoing table, while ⅛ cc. $BaCl_2$ largely increases the urinary secretion, the injection of ¾ cc. in addition to that already present, causes an entire cessation of the flow of urine. In some cases this suppression of the flow of urine is quite abrupt; in other instances it is more gradual, a few drops of urine flowing from the cannula at intervals. As shown in the following experiment, the injection of $CaCl_2$ sometimes counteracts this action of larger doses of $BaCl_2$ and causes the urine to flow again.

5. Rabbit—cannula in bladder; injections intravenous.

Time.	Salts other than NaCl injected.	m/8 NaCl injected in cc.	Urine in cc.
9.55	.....	....	....
10.00	.....	10	1.0
10.15	.....	15	3.4
10.30	.....	15	5.2
10.45	.....	15	5.1
11.00	.....	15	4.8
11.00	1 cc. m/8 $BaCl_2$ + 4 cc. m/8 NaCl		
11.15	.....	10	2.4
11.30	.....	15	0.2
11.45	.....	15	0.3
11.45	5 cc. m/8 $CaCl_2$		
12.00	.....	10	2.0
12.15	.....	15	3.8
12.30	.....	15	4.0

In this case 1 cc. m/8 $BaCl_2$ gradually suppresses the flow of urine, and no trace of the strong diuretic action of barium is seen. And, further, calcium chloride has here an action which seems at first glance entirely opposed to that which it ordi-

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narily has. As shown in the previous experiments, calcium characteristically suppresses the secretion of urine. In this case the flow of urine increases after its administration. These apparent contradictions may be explained in the following way. In discussing the actions of calcium and barium on the intestine, it was pointed out that barium chloride, like the other saline purgatives, affects the intestine in two ways, namely, by increasing the peristaltic movements and by increasing the secretion of fluid into the lumen. Attention was further called to the violent character of the muscular contractions in the intestine caused by barium, which may so constrict the lumen of the intestine that fluid cannot pass from one part to another. It was also shown that calcium to some extent counteracts the action of barium, both on the muscle and on the glands of the intestine. It seems therefore probable that the increase in the flow of urine caused by small doses of barium chloride (1/8 cc. m/8 solution) is due to an increase in the secretory activity of the kidney entirely analogous to that which is produced in the intestine by the same salt. The cessation of the flow of urine, however, which follows the administration of larger doses of barium chloride (1 cc. m/8 solution) is in all probability due to the action of the barium on the muscle coats of the urinary passages, especially those of the calyces and pelvis of the kidney, and those of the ureter. Since all of these various parts of the urinary passages are surrounded by thick circular and longitudinal muscle coats not unlike those of the intestine, it seems conceivable that a strong contraction of these coats, such as barium is capable of causing in the intestine, might effectually shut off the lumen so that no urine could pass. Furthermore, the action of calcium in renewing the flow of urine under these circumstances is quite analogous to its action in suppressing the peristaltic movements or in relieving the constrictions in the intestine caused by barium. The actions of calcium and barium which are shown in Table 5, are on the muscle coats of the urinary passages. It is quite conceivable, however, that this action of calcium may coexist with its characteristic action in diminishing the secretory activity of the kidney. In both the intestine and the urinary apparatus (kidney, and urinary passages) barium stimulates the glandular and the muscular

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tissues to activity. Calcium, on the other hand, uniformly suppresses these activities.

It must be pointed out, however, that the suppression of the flow of urine which follows a relatively large dose of barium chloride cannot always be relieved by calcium. As was found to be true in the intestine, the action of barium is seldom completely counteracted by calcium. In many cases the barium stops the flow of urine entirely so that it is not possible to start it again. This is shown in the following experiment (6), where relatively large quantities of calcium chloride are incapable of reëstablishing the flow of urine. This naturally suggests the idea that the large doses of barium may stop the secretion of urine by injuring the cells of the kidney, or perhaps indirectly by a constricting influence on the blood vessels. These possibilities must be taken into consideration; but the fact that calcium sometimes causes the urine to flow again after it has been inhibited by barium speaks strongly in favor of the theory advanced above, that the inhibiting action of barium on the flow of urine is an action on the muscular tissue of the urinary passages.

6. Rabbit—cannula in bladder; injections intravenous.

Time.	Salts other than NaCl injected.	m/8 NaCl injected in cc.	Urine in cc.
9.50	.....	....	....
10.20	.....	23	....
10.30	.....	20	....
10.40	.....	25	0.8
10.50	.....	28	1.8
11.00	.....	20	2.8
11.10	.....	16	4.6
11.20	.....	10	5.4
11.30	.....	10	5.8
11.40	.....	12	5.2
11.50	.....	15	7.2
11.51	⅔ cc. m/8 $BaCl_2$		
11.55	.....	....	1.0
12.00	.....	15	14.4
12.00	⅓ cc. m/8 $BaCl_2$		
12.05	.....	....	1.6
12.10	.....	14	0.0
12.20	.....	12	0.0
12.30	.....	12	0.0
12.32	5 cc. m/8 $CaCl^2$		
12.40	.....	15	0.0

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Time	Salts other than NaCl injected.	m/8 NaCl injected in cc.	Urine in cc.
12.50	5 cc. m/8 $CaCl_2$	13	....
1.00	.....	10	0.0
1.10	.....	15	0.0
1.20	.....	20	0.0

It will be noticed in this experiment (6) that immediately after the injection of ⅔ cc. m/8 $BaCl_2$ solution there is a marked diminution of the flow of urine, followed within a few minutes by a very considerable increase. This partial cessation of the flow immediately following the injection is due probably to a temporary action of the barium on the muscle coats of the urinary passages. The subsequent increase is the result of the diuretic action of barium on the kidney as described above.

In considering the actions of calcium and barium we must therefore take into account not only their influence on the glandular tissue, but also their effect on the muscular tissue of the body. In all cases these salts are antagonistic in their action; and their influence on the secretory activity of the kidney and on the flow of urine is entirely analogous to their influence on the glandular and muscular activities of the intestine. With regard to its action on the kidney, calcium chloride may be properly termed an *antidiuretic.*

Attention must be again called to the extremely poisonous nature of barium chloride. A subcutaneous injection of 3 cc. m/8 $BaCl_2$ solution is usually sufficient to kill a rabbit. Intravenously it should always be injected with four or five times its volume of m/8 NaCl solution.

CONCLUSIONS.

1. In dogs and rabbits the quantity of urine secreted in a unit of time may for a time be markedly diminished, and in some cases almost entirely inhibited by the introduction of calcium chloride into the circulation.

2. Calcium chloride diminishes not only the normal flow of urine, but also that which is caused by the administration of saline diuretics. For example, the rate of secretion which has been largely increased by the intravenous injection of normal

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salt solution may be temporarily lessened to a marked extent by the introduction of $CaCl_2$ into the blood.

3. In all cases m/8 solutions were used, and m/8 NaCl solution was introduced into the blood at a constant rate throughout the experiments. After a short time the rate of secretion became constant. It was then found in rabbits that the addition of a small quantity of $BaCl_2$ (1/8 cc. m/8 solution) to the blood causes a marked increase in the flow of urine, so that the amount of fluid secreted may considerably exceed that which is introduced into the blood during the same period of time.

4. This action of barium is counteracted by the injection of $CaCl_2$.

5. If a larger quantity of $BaCl_2$ (1 cc. m/8 solution) be added to the blood, the flow of urine ceases and often complete anuria ensues. In some cases the injection of $CaCl_2$ abolishes this inhibitory action so that the urine flows again. Usually, however, the action of barium persists.

6. The fact that barium when given in smaller and in larger doses may thus apparently have opposite effects on the flow of urine may be explained by analogy with its action on the intestine. Barium chloride causes not only an increase in the secretion of fluid into the intestine, but also active peristaltic movements, and violent local constrictions of the intestine. Similarly, very small doses of $BaCl_2$ increase the secretory activity of the kidney. It seems probable, however, that the cessation of the flow of urine which follows the injection of larger quantities of the salt is due, not to an inhibition of secretion, but to the action of the barium on the muscular coats of the urinary passages, especially those of the calyces and pelvis of the kidney and those of the ureter. This action would bring about a constriction of the tubes and a closure of the lumen. The fact that calcium counteracts both effects of the barium supports this explanation.

7. The influence of calcium and barium on the flow of urine is in every way analogous to their action on the intestine, which I have previously described. The suppression of the urinary secretion by calcium is also analogous to the suppression of

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twitchings in voluntary muscles by calcium, which has been described by Loeb.

In conclusion, it is a pleasure to thank Professor Loeb for the interest which he has taken in these experiments. I am indebted also to Dr. Theo. C. Burnett, who has assisted me in many of the experiments.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 4, pp. 43-46. December 9, 1904

NOTE ON THE GALVANOTROPIC REACTIONS OF THE MEDUSA POLYORCHIS PENICILLATA A. AGASSIZ.[1]

BY

FRANK W. BANCROFT.

(From the Rudolph Spreckels Laboratory of the University of California.)

Comparatively few papers on the galvanotropic reactions of coelenterates have been published and so far as I know there are only two bearing directly on the questions here considered. The first is by Pearl[2], who finds that when any, except the very strongest, galvanic currents are passed transversely through hydra the animal contracts most strongly on the anode side, so that the free end—which may be either oral or aboral—swings around and points toward the anode. The tentacles, however, behave differently. With weak currents only those tentacles which are parallel to the current lines contract, but of these the one towards the cathode has a tendency to contract most strongly. When the whole animal has become oriented the tentacles curve slightly so as to become concave on the side towards the cathode. The second observation is by Greeley, and will be considered in detail later on.

The tentacles and manubrium of Polyorchis penicillata, which occurs aboundantly in San Francisco Bay during certain seasons of the year, furnish excellent material for the demonstration of galvanotropic reactions, responding to the current in some respects like the tentacles of hydra, but with greater distinct-

[1]Reprinted from *Journal of Experimental Zoölogy*, 1904, No. 2, p. 289.

[2]PEARL: 1901, Studies on the Effects of Electricity on Organisms. II.—Reactions of Hydra to the Constant Current. *Amer. Jour. Physiol.*, Vol. V, pp. 301-320.

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ness. The method of experimentation consisted in cutting the medusæ in various ways and placing the pieces in a trough of sea water through which the galvanic current was conducted with non-polarizable electrodes. The current strength varied from 25 to 200 δ. The responses were usually distinct with 25 δ, but became more decided as the current was increased.

If a meridional strip passing from the edge on one side through the center of the bell to the other edge be prepared and the current passed through it transversely, tentacles and manubrium turn and point toward the cathode (Fig. 1). A reversal of the

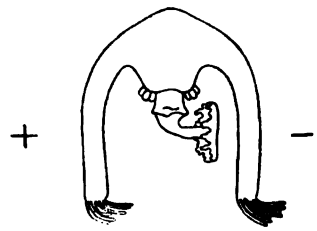

FIG. 1.

current initiates a turning of these organs in the opposite direction, which is usually completed in a few seconds. This can be repeated many times and the tentacles continue to respond after hours of activity. The manubrium, however, tires sooner and fails to respond. If the strip is placed with its subumbrellar surface upwards and extended in a straight line parallel to the current lines, the making of the current causes the tentacles at the anode end to turn through an angle of 180 degrees and point

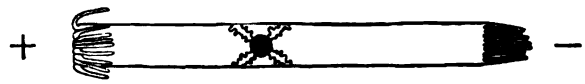

FIG. 2.

towards the cathode. The tentacles at the cathode end become more crowded together, reminding one of the tip of a moistened paint brush, and also point more directly towards the cathode (Fig. 2). The experiment may be varied in still other ways by cutting smaller or larger pieces from the edge of the swimming bell, but the response is always the same. The tentacles wherever possible, and to a less extent, the manubrium, bend so as to point toward the cathode. The response depends in no way upon the connection of these organs with the swimming

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bell, muscles or nerve-ring, for it is obtained equally well with isolated tentacles and pieces of tentacles. Isolated tentacles when placed transversely to the current lines curve so as to assume a more or less complete U-shape, with their concave side

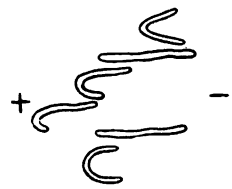

FIG. 3.

toward the cathode (Fig. 3). When placed parallel to the current the tentacles do not curve (Fig. 3, *a*).

If the tentacles are relaxed the making of the current causes them to contract rapidly. Subsequently they turn their concave side towards the cathode, and remain contracted for a considerable period. But if the current is continued long enough through the isolated tentacles a partial relaxation comes on, which is suddenly followed by another rapid contraction; so that we have, as this process repeats itself, a slow and irregular rhythmic contraction, caused as in the case of the quiescent frog's ventricle by the constant flow of the galvanic current. If the current is

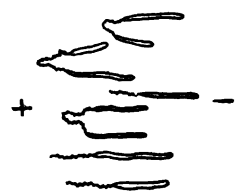

FIG. 4.

continued still longer in some cases a local anodal relaxation occurs, and the isolated tentacles then have the apearance of Fig. 4. As the figure shows, this relaxation is at the bend of the U in the curved tentacles and at the anodal end in those which were parallel to the current lines and did not curve.

It is evident that these phenomena lend themselves very nicely to Loeb's[1] explanation of galvanotropism, which he con-

[1] J. LOEB: 1897, Zur Theorie der physiologischen Licht und Schwerkraftwirkungen. *Pflüger's Archiv.* Bd. 66, p. 440.

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siders depends on similar changes in the tension of associated groups of muscles. The constant flow of the current brings about an increase of tension on the cathodal side of tentacles and manubrium, as a result of which this part is more strongly contracted than the anodal portion. When the tentacles are exhausted the anodal part may even be completely relaxed.

Greeley[2] has stated that when "Gonionemus was exposed to the constant current, rhythmical contractions began always on the cathodal side when the medusa was immersed in normal sea water, but that the contractions began on the anodal side in acidulated sea water." A series of experiments was made on Polyorchis to test its behavior in acid and alkaline sea water, but as long as the tentacles were sufficiently uninjured so that they responded at all to the current, they behaved as above described, no matter what the reaction of the water. This influence of acid and alkaline media on the contraction of the muscles, was also tested, but Greeley's results could not be confirmed. Usually a change in the reaction of the sea water made no difference, and even when it did the change in the electrical response was sometimes in one direction and sometimes in another, so that no significance could be attached to it.

As a rule the muscles of a meridional strip of Polyorchis do not behave toward the galvanic current as described by Greeley for Gonionemus in normal sea water; for the place of maximum response is the anode. It is here that the contractions usually start and here that the most rapid rate of the rhythmic contractions is usually seen. But there is such an abundant opportunity for stimulation at secondary cathodes that I am not yet prepared to say that we have here an exception to Pflüger's law.

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[2] F. TRELEASE: 1903, Report of a meeting of the Academy of Sciences of St. Louis. *Science, N. S.*, Vol. XVIII, p. 753.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 5, pp. 47–64 January 7, 1905

THE ACTION ON THE INTESTINE OF SOLUTIONS CONTAINING TWO SALTS.

BY

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In previous papers[1] I have discussed the action of various salts on the intestine when injected subcutaneously or intravenously, or directly applied to the peritoneal surfaces of the intestine. It was shown that many sodium salts, especially sodium citrate, sulphate, fluoride and phosphate and barium chloride increase the peristaltic activity of the intestine when administered by any of the methods mentioned, and that in addition to this the secretory activity of the intestine could be also increased by sodium citrate or by barium chloride. It was shown also that these activities could be suppressed by the administration of calcium chloride, and to some extent also by magnesium chloride. It was further pointed out that these effects are analogous to the production and suppression of rhythmical twitchings in the voluntary muscles of the frog by the same salts, which had already been demonstrated by Loeb.[2] In a further series of experiments it was shown that muscular movements as well as some secretory activity are produced in loops of the intestines entirely removed from the body and immersed in solutions of various salts.

[1] MACCALLUM, J. B.: *University of California Publications, Physiology*, 1903, I, p. 5, p. 115, p. 125. *American Journal of Physiology*, 1903, X, p. 101, and 1904, X, p. 259. *Pflüger's Archiv.*, Bd. 104, p. 421, 1904.

[2] LOEB, J.: *Festschrift f. Fick*, 1899: *Pflüger's Archiv.*, 1902, XCI, p.248.

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The following is a description of a large number of experiments made in the study of certain differences which exist between the behavior of loops of the intestine which retain their normal connection in the body, and loops which have been removed from the body, when subjected to the action of the same solution. In connection with this there were observed also marked differences between the behavior of loops removed from the body and immersed in solutions of certain salts alone, and the behavior of similar loops in solutions in which two salts were mixed. For example, loops placed in lithium chloride alone or in calcium chloride alone behave entirely differently from loops placed in a mixture of lithium chloride and calcium chloride.

The results of these experiments may be described under the following headings:

I. Methods.

II. Action of LiCl, NaCl, KCl, $CaCl_2$ and $MgCl_2$ on loops of the intestine normally connected in the body, and the effects produced by isolating these loops from the central nervous system.

III. Behavior of loops of intestine removed from the body in solutions of LiCl, NaCl, KCl, $CaCl_2$ and $MgCl_2$, and in mixtures of these solutions.

IV. Conclusions.

I.—Methods.

Small rabbits were used for these experiments, weighing 1200-1500 g. They were narcotized by a subcutaneous injection of 4-5 c.c. 1% morphine hydrochlorate solution. The study of the action of various solutions on the intact intestine was made either by opening the abdomen under the surface of the solution in a large vessel, so that the intestines come immediately in contact with the solution and are not at all exposed to the air, or by opening the abdomen in the ordinary way and applying the solutions directly to the peritoneal surfaces or allowing the loops to lie in a beaker containing the solution to be tested. All of the solutions were kept at body temperature.

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In removing the loops from the body in the other experiments the intestines were exposed and a loop about 30-40 cm. in length was isolated by ligatures. By means of a needle and thread the blood vessels supplying this loop were carefully tied and the loop rapidly excised. It was then cut into a number of pieces, usually four, which were transferred with as little handling as possible to the beakers containing the solutions to be tested. These beakers were kept in a water bath at 39.5°C. It is desirable in these experiments to use loops which contain no foeces, since unknown substances in the foeces might go into solution and disguise the true action of the salt being tested. For this reason the upper part of the small intestine was principally used, since in the rabbit it is almost always empty. For any set of experiments the loops must all come from the same rabbit, for there exist considerable differences in irritability in different animals. On account of these differences it is necessary to have control experiments in the case of each rabbit used. Loops also which have been exposed to the air are usually more irritable than those immediately removed from the body.

The solutions were made up with distilled water ,and Kahlbaum's preparations. A concentration of m/6 was assumed to be roughly isosmotic with the tissue fluids in all cases.

II.—Action of LiCl, NaCl, KCl, $CaCl_2$ and $MgCl_2$ on loops of the intestine normally connected in the body, and the effects produced by isolating these loops from the central nervous system.

Sodium chloride in m/8 or m/6 solution may be injected in fairly large quantities under the skin or into the veins of a rabbit without causing an increased peristaltic activity. If also the abdominal cavity of a rabbit be opened beneath the surface of m/6 NaCl solution at body temperature, the normal movements of the intestine are not markedly increased by contact with the salt solution. This was observed also by Van Bram-Houckgeest[1] and was used as the basis of a long series of experiments which he made on the effect of section of the intestinal

[1] *Pflüger's Archiv.* Bd. 6, 1872; and Bd. 8, 1874.

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nerves. He found that section of the splanchnic nerves caused a very marked increase in the peristaltic movements of the intestine when it was submerged in NaCl solution. This effect he regarded as due to the vasomotor functions of these nerves. It had been long before stated by Claude Bernard[1] that section of the cord below the origin of the phrenic nerve caused active movements to appear in the intestine. This was confirmed by Pflüger, who applied the term inhibitory nerves of the intestine to the splanchnic nerves, since he found that stimulation of their peripheral cut ends caused movements of the intestine to cease. It was further shown by Claude Bernard that the secretion of the intestine is increased by destruction of the coeliac ganglia; and Moreau[2] demonstrated the fact that section of the mesenteric nerves causes a large increase in the fluid secreted into the intestine.

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I have confirmed these results and have found that loops which are quiet when the abdomen is opened remain quiet when placed in m/6 NaCl solution. If, however, the loops have become active through exposure to the air, these movements continue when the loops are placed in the salt solution. In any case section of the cord in the cervical region, or section of the splanchnic nerves or clamping of the nerves and blood vessels supplying the loops, causes a very marked increase in the peristaltic activity of these loops.

Solutions of LiCl (m/6) poured on the loops of intestine do not increase the peristaltic movements. Loops which have been exposed to the air continue their activity for some time in this solution. Separation of the loop from the central nervous system causes it to become very active, just as in the case of m/6 NaCl.

A drop of m/6 KCl placed on the peritoneal surface of a loop causes it to contract violently under the drop. This contraction spreads to the rest of the loop, and a very small quantity of this solution is sufficient to cause the whole intestine to become very active. The action of KCl is somewhat similar in this respect to

[1] Leçons sur la physiologie et la pathologie du système nerveux. Paris, 1858, T. 1, p. 362-365.

[2] *Centralblatt f. d. medicin. Wiss.*, 1868, S. 209.

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that of $BaCl_2$ which has been described in previous papers. A solution of KCl as dilute as m/100 will cause marked peristaltic movements when applied to the peritoneal surface of the intestine. These movements may be inhibited by the subsequent application of m/6 $CaCl_2$ solution.

As has been already described, m/6 solution of $CaCl_2$ causes no movements when applied to the intestine or when administered subcutaneously or intravenously. On the contrary, it inhibits movements which are already present. When normal loops are left in contact with such a solution they remain perfectly quiet. If the cord or the splanchnic nerves be cut the loops become active for a moment, and move slowly in a swinging rhythmical manner. Within a minute however they again come to rest. The same is true of loops removed from the body. Solutions of $MgCl_2$ have an action similar to that of $CaCl_2$.

If a very small quantity of $CaCl_2$, *e.g.*, 4-5 c.c. m/6 solution be added to 50 c.c. m/6 LiCl in which a loop is moving slightly, the movements seem to be somewhat increased. If however a larger quantity of $CaCl_2$, *e.g.*, 40-50 c.c. m/6 solution be added, the loop becomes entirely inactive.

III.—Behavior of loops of intestine removed from the body in solutions of LiCl, NaCl, KCl, $CaCl_2$ and $MgCl_2$, and in mixtures of these solutions.

(a) LiCl and $CaCl_2$. A loop of intestine removed from the body and placed in a beaker of m/6 LiCl solution at 39.5°C varies somewhat in its behavior according to the irritability of the animal, and the rapidity of its removal. All similar loops taken at the same time from the same animal behave in the same way under similar conditions. In some cases the loop shows no movements whatever, but remains entirely at rest from the time of its being placed in the solution. The majority of loops move rhythmically for ¼ to ½ minute after being put into the solution. These movements are similar to those seen in the loops which are still connected with the body. They are quiet and regular, and not at all convulsive in character. When the loop has come to rest it remains so until the end of the experiment.

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In a certain number of cases the loop when transferred to m/6 LiCl shows active movements resembling those which were seen in loops in the same solution after the spinal cord had been cut. These may last 5-10 minutes but seldom longer. They gradually cease and at the end of 15 minutes the loop is entirely at rest. These movements occur more often in loops which have been exposed for a relatively long time to the air, and in loops which contain foeces. It is probable that the movements which are seen in LiCl are merely the continuation of those movements which are produced by the separation of the loop from the central nervous system. The nature of the LiCl solution seems to be in some way unsuitable for their long duration. This is to be compared with the behavior of similar loops in m/6 NaCl as previously described,[1] where the movements persist for 40-60 minutes.

A loop excised from the same rabbit and placed in m/6 $CaCl_2$ solution at 39.5°C remains in the great majority of cases quite motionless from the first. In a few instances slight movements are present immediately after the loop is submerged, but after a few seconds these disappear, and the loop remains at rest. After 25-40 minutes it is not uncommon to observe the loop slowly straighten out, and at the end of this time the length of the loop is found to be much less than it was at first. This seems to be due to a slow contraction of the longitudinal muscle layer, so slow that no movement can be observed. A marked difference is seen in the position and shape of the loops placed in LiCl and in $CaCl_2$ after they have both come to rest. That in LiCl is relatively long and is coiled up in a circle; that in $CaCl_2$ is about half its original length and is almost straight.

A loop removed from the body of a rabbit and placed in 50 c.c. m/6 LiCl + 5 c.c. m/6 $CaCl_2$ at 39.5°C behaves in a manner entirely different from similar loops placed in either LiCl or $CaCl_2$ alone. On being first placed in the solution it shows practically no movements at all. Even in those cases where the control loop in LiCl is active at first, the corresponding loop in the mixture of LiCl and $CaCl_2$ shows no movements. It remains

[1] J. B. MacCallum: *University of California Publications, Physiology*, Vol. I, No. 13, 1904.

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perfectly quiet for 10-15 minutes. Then a remarkable change takes place. Sharp constrictions appear suddenly in one or more places in the loop. These are followed a second or two later by violent contractions which cause the loop to coil upon itself in a most active manner. These contractions, which resemble somewhat those caused by barium chloride in the body, follow one another so rapidly that the loop is turned and twisted into knots. This extreme activity persists for 30 to 45 minutes, sometimes for an hour, while during all this time the control loops in LiCl and in $CaCl_2$ are entirely motionless. The movements are not at all to be compared with those slight movements which were described as occurring at the beginning of the experiment in pure LiCl solution. They are of entirely different character and order of magnitude. The course of such an experiment may be seen in the following table:

TABLE I.

Time	50 c.c. m/6 LiCl	50 c.c. m/6 LiCl + 5 c.c. m/6 $CaCl_2$	50 c.c. m/6 $CaCl_2$
Loops placed in solutions at			
10:05	no movements	no movements	no movements
10:10	"	"	"
10:15	"	"	"
10:19	"	violent movements begin	"
10:25	"	very active movements	"
10:30	"	"	"
10:45	"	"	"
10:50	"	movement less active	"
11:00	"	" very slight	"
11:15	"	" almost stopped	"
11:20	"	no movements	"

These results are somewhat varied by varying the proportions of LiCl and $CaCl_2$. The characteristic contractions may be obtained with as low a concentration of $CaCl_2$ as 50 c.c. m/6 LiCl

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+ ½ c.c. m/6 $CaCl_2$. In this case however the movements though of characteristic nature persist for only 5-10 minutes, as shown in the following table:

TABLE II.

Time	50 c.c. m/6 LiCl	50 c.c. m/6 LiCl + 5 c.c. m/6 $CaCl_2$	50 c.c. m/6 LiCl + 2 c.c. m/6 $CaCl_2$	50 c.c. m/6 LiCl + ½ c.c. m/6 $CaCl_2$
Loops placed in solutions at				
10:12	slight mov'm'ts	no movements	no movements	no movements
10:18	"	mov'm'ts begin	"	mov'm'ts active
10:20	"	very active movements	active mov'm'ts	" ceasing
10:25	no movements	" "	" "	no movements
10:30	"	" "	" "	"
10:35	"	" "	" "	"
10:50	"	movements less active	movements less active	"
11:00	"	no movements	no movements	"

In this case movements were seen also in the loop in pure LiCl solution. These are probably the continuation of movements caused by separating the loop from the central nervous system. At first sight it would seem that the movements seen in mixtures of LiCl and $CaCl_2$ are merely the movements present in LiCl delayed by the presence of the $CaCl_2$. That this is not the case may be easily shown. In the first place they are many times more powerful than the movements in pure LiCl and of an entirely different character, being violent and convulsive. Further, if they were the movements seen in LiCl, only delayed by the $CaCl_2$, they should be more active in the solutions containing the least $CaCl_2$. The opposite is the case. In 50 c.c. LiCl + ½ c.c. $CaCl_2$ the movements are relatively slight as compared with those in 50 c.c. LiCl + 5 c.c. $CaCl_2$. A further experiment shows this still more clearly. A loop placed in 50 c.c. m/6 LiCl was allowed to come to rest, and was left in this solution for 10 minutes. No movements whatever were seen at this time. There were then added 5 c.c. m/6 $CaCl_2$ to the LiCl solution containing

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TABLE III.

Time	50 c.c. LiCl	40 c.c. LiCl + 10 c.c. $CaCl_2$	30 c.c. LiCl + 20 c.c. $CaCl_2$	25 c.c. LiCl + 25 c.c. $CaCl_2$	20 c.c. LiCl + 30 c.c. $CaCl_2$	5 c.c. LiCl + 50 c.c. $CaCl_2$	50 c.c. $CaCl_2$
10:55	no movements	no movements	no movements	no movements	no movements	no movements	no movements
11:00	"	"	"	"	"	"	"
11:12	"	"	"	"	movements begin	"	"
11:15	"	movements begin	"	movements begin	" continue	"	"
11:18	"	" continue	movements begin	" continue	" "	mov'm'ts begin	"
11:25	"	" "	" continue	" "	" "	" continue	"
11:30	"	" "	" "	" "	" "	no movements	"
11:40	"	" "	" "	" "	" "	"	"
11;45	"	" "	" "	" "	no movements	"	"
11:50	"	no movements	" "	" "	" "	"	"
12:00	"	"	no movements	no movements	" "	"	"

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the motionless loop. Within one minute the loop became violently active in the characteristic way described above. The activity continued for nearly an hour.

Similar results were obtained with mixtures containing a larger proportion of $CaCl_2$. With 25 c.c. m/6 LiCl + 25 c.c. m/6 $CaCl_2$ the movements usually cease in about 20 minutes. With greater concentration of $CaCl_2$, *e.g.*, 5 c.c. m/6 LiCl + 50 c.c. m/6 $CaCl_2$, the movements appear later and last only 5-10 minutes. The loops with relatively much $CaCl_2$ come to rest in the shape characteristic of loops in pure $CaCl_2$; they become relatively short and are found straightened out at the end of the experiment. In the experiments where relatively much LiCl is present in the mixture the loops are found much coiled and twisted and are not shortened. The effect of various proportions of these two salts is shown in table III.

In the following table is further shown the relative behavior of loops in mixtures which contain much LiCl and those which contain much $CaCl_2$.

TABLE IV.

Time.	50 c.c. LiCl	50 c.c. LiCl + 5 c.c. $CaCl_2$	50 c.c. $CaCl_2$ + 5 c.c. LiCl	50 c.c. $CaCl_2$
11:14	Length of loop 10 cm.	Length of loop 10 cm.	Length of loop 10 cm.	Length of loop 10 cm.
11:14	no movements	no movements	no movements	no movements
11:20	"	very active movements	"	"
11:35	"	" "	mov'm'ts begin	"
11:40	"	" "	" slow	"
11:50	"	" "	no movements	"
12:00	"	" "	"	"
12:05	"	no movements	"	"
12:10	Length about 8 cm.	Length about 8 cm.	Length about 4 cm.	Length about 4 cm.

From these experiments it is to be observed that loops of intestine removed from the body of a rabbit are relatively inactive when placed in isotonic solutions of pure LiCl, or pure $CaCl_2$.

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When however they are placed in a mixture of these two solutions a very marked activity appears. The nature of this activity is somewhat influenced by the relative concentrations of LiCl and $CaCl_2$ in the mixture.

The slight movements which sometimes appear when a loop is first placed in LiCl solution are probably a continuation of the normal movements of the intestine which have been accentuated by the separation of the loop from the central nervous system. They do not appear in a mixture of LiCl and $CaCl_2$. The $CaCl_2$ inhibits them here just as it does in the body. The duration of the movements depends on the irritability of the loop and on the nocuous or innocuous nature of the solution into which it is placed. In m/6 NaCl, as has been mentioned, they persist for 40-60 minutes, a fact which is possibly due to the innocuous nature of the NaCl solution. In solutions of other salts, *e.g.*, $BaCl_2$, NaF, Na_2SO_4, Na citrate, etc., the movements which are at first present cease very rapidly. It cannot be said that the NaCl solution causes the movements in this case, nor that the solutions of other salts inhibit them. The NaCl solution seems to be in some way a medium better suited, perhaps less injurious, for the activity of the tissues.

The muscular contractions which however appear in mixtures of LiCl and $CaCl_2$ and do not appear in LiCl or in $CaCl_2$ alone cannot be said to be a continuation of the movements caused by separating the loop from the central nervous system. They evidently depend in some way on the absorption of the salts. One is tempted to suggest that the LiCl solution has a toxic or inhibitory action on the loop, and that the addition of $CaCl_2$ solution counteracts this. This however does not explain the violent contractions, which are like nothing that can be produced by either LiCl or $CaCl_2$. Also if the LiCl solution were toxic, it is difficult to imagine that the loop could be suddenly restored to activity as described above by the addition of a small quantity of $CaCl_2$ after it had lain in pure LiCl solution for 15 to 20 minutes. It cannot, on the other hand, be supposed that the $CaCl_2$ acts as a stimulating agent, for all evidence goes to show that $CaCl_2$ has quite the opposite action under all other conditions. The action of $CaCl_2$ in this instance is suggestive of the action of a catalyser,

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the addition of which enormously hastens some chemical reaction. It is possible that the muscular activity in this case depends on chemical reaction which is hastened neither by LiCl nor by $CaCl_2$, but by a combination of these two, or perhaps by an intermediate product. Whatever may be the explanation of these phenomena, the fact remains, and is easy of demonstration, that an effect is produced by a combination of LiCl and $CaCl_2$ which is entirely different from the action of either LiCl or $CaCl_2$ alone.

(b) Action of KCl alone and in combination with other salts. As mentioned above, m/6 KCl solution applied to the peritoneal surface of loops of the intestine causes these loops to become very active. This activity is largely inhibited by the subsequent application of m/6 $CaCl_2$ solution. A loop removed from the body and placed in m/6 KCl at body temperature moves actively for a few seconds, but rapidly comes to rest and remains quiet. In much more dilute solutions of KCl, *e.g.*, m/50, the loops come to rest also in a few seconds. This, however, also takes place in distilled water. The behavior of loops in pure $CaCl_2$ solution has been described above.

If, however, a loop be placed in a mixture of 60 c.c. m/6 $CaCl_2$ + 6 c.c. m/6 KCl a very different result is obtained. During the first 20 to 25 minutes following the immersion of the loop in this mixture practically no movements are seen. Then, however, active movements appear and persist for 10 to 15 minutes, and sometimes longer. These movements are not so active as those described in LiCl + $CaCl_2$ and are of a slower, less convulsive character. Control loops placed in 50 c.c. distilled water + 6 c.c. m/6 KCl and in 50 c.c. m/6 $CaCl_2$ show no movements corresponding with these. The behavior of loops in various mixtures of m/6 KCl and in m/6 $CaCl_2$ is shown in table V.

This seems at first sight to be quite analogous with the effects produced by mixtures of LiCl and $CaCl_2$. KCl, however, has in itself a powerful action on the intestine in the body, and the fact that loops removed from the body and placed in solutions of KCl soon become inactive is due probably to the poisonous nature of KCl. In the body it is constantly absorbed and diluted by the blood as it is carried away from the place of application. It therefore never reaches here the concentration which acts on

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a loop removed from the body and placed in a m/6 solution. When the concentration is diminished and the isolated loop is placed, for example, in 50 c.c. distilled water + 6 c.c. KCl there is still another factor to be considered, *i.e.*, the diminished osmotic pressure of the solution; and, as mentioned before, loops soon come to rest in distilled water. If, in place of this solution, there is used a mixture of 50 c.c. m/6 LiCl + 6 c.c. m/6 KCl, or 50 c.c. m/6 NaCl + 6 c.c. m/6 KCl, there are produced active movements which are not similar to those present in either LiCl or NaCl alone. These movements seem to be due directly to the KCl. The behavior of loops in various mixtures of m/6 LiCl and m/6 KCl is shown in table VI.

Here the loop in 50 c.c. LiCl + 3 c.c. KCl showed more powerful movements than did the one in pure LiCl. The loop in 50 c.c. LiCl + 10 c.c. KCl, however, became extremely active, resembling in its convulsive movements the loops described in LiCl + $CaCl_2$. Loops in mixtures containing a greater quantity of KCl soon came to rest.

(c) Action of NaCl and $CaCl_2$. As already mentioned, loops of intestine removed from the body and placed in pure m/6 NaCl solution at 39.5°C show regular muscular movements which continue for 30 to 60 minutes. These movements are not at all convulsive or violent in nature but resemble the normal peristaltic movements in the body. When a loop of intestine still normally connected in the body is allowed to rest in a beaker of m/6 NaCl solution, or when the abdomen is opened under the surface of this solution, only slight movements are usually seen. If the cord be cut or the loop suddenly cut away from the body and allowed to fall into the solution, it becomes much more active. These are the movements which are seen when a loop is excised as described and placed in the m/6 NaCl solution. The behavior of loops in m/6 $CaCl_2$ has already been described.

When, however, a loop is excised and placed in 50 c.c. m/6 NaCl + 10 c.c. m/6 $CaCl_2$ the behavior is quite different. There are no movements whatever to be seen at first. The loop remains quiet for about 10 minutes. The movements which are seen from the beginning in the control loop in pure NaCl solution have apparently been inhibited by the $CaCl_2$ present in the mixture,

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TABLE V.

Time	50 c.c. dist. H_2O + 6 c.c. KCl	50 c.c. $CaCl_2$	50 c.c. $CaCl_2$ + 1 c.c. KCl	50 c.c. $CaCl_2$ + 6 c.c. KCl	50 c.c. $CaCl_2$ + 15 c.c. KCl	25 c.c. CaCl + 25 c.c. KCl	50 c.c. KCl
10:05	slight mov'm'ts	no movements	no movements	no movements	no movements	momentary contractions	momentary contractions
10:07	no movements	"	"	"	"	no movements	no movements
10:17	"	"	"	"	active mov'm'ts	"	"
10:25	"	"	"	"	"	"	"
10:28	"	"	"	active mov'm'ts	"	"	"
10:30	"	"	movements	"	no movements	"	"
10:35	"	"	"	"	"	"	"
10:38	"	"	no movements	no movements	"	"	"

TABLE VI.

Time	50 c.c. LiCl	50 c.c. LiCl + 3 c.c. KCl	50 c.c. LiCl + 10 c.c. KCl	50 c.c. LiCl + 20 c.c. KCl	50 c.c. KCl
11:00	mov'm'ts fairly active	mov'm'ts fairly active	movements fairly active	movements active	moventary contractions
11:10	slight movements	slight movements	slight movements	almost at rest	no movements
11:15	"	"	extremely active	no movements	"
11:20	"	increased movement	" "	"	"
11:25	no movements	"	" "	"	"
11:30	"	no movements	less active	"	"
11:35	"	"	no movements	"	"

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just as $CaCl_2$ solution inhibits such movements in the body. At the end of 10 minutes, however, the loop gradually becomes very active, and these contractions are not of the quiet, even character of those seen in the loop in pure NaCl. They resemble more closely those which have been described in loops immersed in mixtures of LiCl and $CaCl_2$. Sharp constrictions and quite violent twistings occur, and the loop becomes much more active than that in NaCl. The result is almost the same as described with mixtures of LiCl and $CaCl_2$, except that in the latter case the onset of the contractions is more sudden, and the control loop in pure LiCl comes to rest more quickly. The onset of the contractions in NaCl + $CaCl_2$ is gradual and the activity increases and persists for 30 to 40 minutes, sometimes for an hour. When the concentration of $CaCl_2$ in the mixture is relatively great this effect is not obtained. Thus here also, as in the case of mixtures of LiCl and $CaCl_2$, there is produced by a mixture of two salts an activity which is much greater than that produced by either salt alone. This effect is shown in table VII, in which all the solutions are m/6.

It thus seems that the presence of $CaCl_2$ inhibits the movements which are first present in a loop placed in NaCl solution. When added in small quantities, e.g., not more than 10 c.c. m/6 $CaCl_2$ to 50 c.c. m/6 NaCl it produces very violent movements, such as are never seen in pure NaCl solution. When, however, it is added in greater proportion than this, *e.g.,* 20 or more c.c. m/6 $CaCl_2$ to 50 c.c. m/6 NaCl, all movements are inhibited. The explanation of this is not clear.

If a loop be placed in a mixture of LiCl and NaCl in equal parts movements appear such as are seen in pure NaCl solution but do not persist for such a long time. In the mixture of these two salts no such effect is produced as has been described in mixtures of LiCl and $CaCl_2$ and of NaCl and $CaCl_2$. Mixtures also of LiCl and KCl owe their action probably to the direct influence of the KCl. Mixtures of $CaCl_2$ and $MgCl_2$ produce no movements. Loops lie quiet in such mixtures, just as they lie at rest in pure $CaCl_2$ or pure $MgCl_2$. One is tempted to suggest, from these experiments, that mixtures of chlorides of two univalent metals do not produce the great activity described nor do mix-

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TABLE VII.

Time	50 c.c. NaCl	50 c.c. NaCl + 5 c.c. $CaCl_2$	50 c.c. NaCl + 10 c.c. $CaCl_2$	50 c.c. NaCl + 20 c.c. $CaCl_2$	50 c.c. NaCl + 40 c.c. $CaCl_2$	50 c.c. $CaCl_2$
10:35	quite active	no movements	no movements	no movements	no movements	no movements
10:40	"	"	"	"	"	"
10:45	"	slight movements	slight movements	"	"	"
10:50	less active	"	"	"	"	"
10:55	still active	very active	very active	"	"	"
11:00	"	extremely active	extremely active	"	"	"
11:15	"	"	"	"	"	"
11:30	almost stopped	"	"	"	"	"
11:45	no movements	movements quieter	movements quieter	"	"	"
11:50	"	no movements	no movements	"	"	"

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tures of chlorides of two bivalent metals. But this activity is produced by mixtures of chlorides of one monovalent and one bivalent metal. The evidence of these experiments is not, however, sufficient to justify such a statement, and it may be accidental that these active movements are produced conspicuously by LiCl + $CaCl_2$, NaCl + $CaCl_2$ and KCl + $CaCl_2$; while they are not produced by LiCl + NaCl, LiCl + KCl, NaCl + KCl, or $CaCl_2$ + $MgCl_2$.

IV.—Conclusions.

1. The activity of loops of intestine with blood and nerve supply in a rabbit is not increased by submerging them in m/6 NaCl solution at 39.5°C. If, however, the spinal cord or the splanchnic nerves be cut, these loops show very active peristaltic movements. This occurs also in m/6 LiCl; but in m/6 $CaCl_2$ the movements soon cease and the loop comes to rest.

2. Loops of intestine removed entirely from the body and placed in m/6 NaCl at 39.5°C move actively and remain active for 40-60 minutes. In m/6 LiCl the movements are slighter and cease in from 5 to 15 minutes; sometimes they are absent from the first. In m/6 $CaCl_2$ such loops remain inactive from the beginning.

3. If three loops be excised from the body and placed respectively in 50 c.c. m/6 LiCl, 50 c.c. m/6 $CaCl_2$, and 50 c.c. m/6 LiCl + 5 c.c. m/6 $CaCl_2$, the loop in LiCl will show slight movements, which soon disappear; the loop in $CaCl_2$ will show no movements at all; while the loop in the mixture of LiCl and $CaCl_2$ will show, after 10 to 15 minutes, most violent contractions, which persist for 30 to 40 minutes.

4. If a loop be placed in 50 c.c. m/6 LiCl for 10 to 15 minutes, until all movements have ceased, and 5 c.c. m/6 CaCl be then added to the solution, the loop becomes extremely active and continues to move for 30 or 40 minutes.

5. If loops be placed in mixtures of LiCl and $CaCl_2$ in which the proportion of $CaCl_2$ is relatively very great, all movements are inhibited.

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6. A loop placed in m/6 NaCl moves actively for a considerable time; one placed in m/6 $CaCl_2$ does not move at all. A loop placed in 50 c.c. m/6 NaCl + 10 c.c. m/6 $CaCl_2$ shows no movements for the first 10 to 15 minutes; it then becomes extremely active, and exhibits much more violent contractions than are ever seen in pure NaCl.

7. Solutions of KCl applied to loops of intestine in the body cause very active muscular contractions. These may be inhibited by $CaCl_2$. When loops are excised and placed in m/6 KCl they contract powerfully for a moment and then come to rest. A loop placed in 50 c.c. m/6 $CaCl_2$ + 10 c.c. m/6 KCl shows no movements at first, but after 20 to 25 minutes active contractions appear and continue for 10-15 minutes.

8. KCl added to solutions of NaCl or LiCl in which loops are placed merely increases the activity of the loop.

9. No movements are obtained by placing loops in mixtures of $CaCl_2$ and $MgCl_2$.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 6, pp. 65-70. January 7, 1905

THE ACTION OF PURGATIVES IN A CRUSTACEAN *(Sida crystallina)*.

BY

JOHN BRUCE MACCALLUM.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

In connection with the study of the action of purgatives in higher animals the question arose as to whether or not these materials have a similar influence on the intestine of much simpler forms. A class of animals peculiarly adapted for the study of this question was found in the Cladocera, many species of which are abundant in fresh-water pools. The transparency of the body, and the clearly outlined intestine afford an excellent indicator of the action of various solutions. The form which was made use of in the following experiments was *Sida crystallina,* which is relatively large and particularly transparent. It varies in length from 1 to 1.5 mm., but sometimes measures 2 to 2.5 mm. The alimentary canal is without convolutions, and passes in a somewhat curved direction throughout the body. The oesophagus enters at a sharp angle a dilated portion of the gut, which represents the stomach. From the anterior end of this structure there open two diverticula or coeca. These seem to be of a glandular nature, and are sometimes spoken of as digestive glands. They are usually filled with a greenish fluid. The intestine extends in a fairly straight line to the post-abdomen, where it turns sharply at right angles a short distance in front of the anus. The intestine is always filled with brownish foeces, which are normally expelled in small quantities, only at long intervals. The stomach is usually filled with greenish material. Slight per-

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istaltic waves are commonly to be seen in the lower part of the intestine. The other organs may be observed in this animal with equal clearness. The heart may be seen beating rapidly (200 or more times per minute), and definite streams of blood corpuscles may be watched. There exists, however, no closed blood vascular system; the blood currents are directed by membranous partitions.

The animals were studied in groups of five or more. Such groups were placed in various solutions in watch-glasses, and observed continuously with a low power of the microscope. It was found that pilocarpine, barium chloride, sodium citrate, sulphate and fluoride caused an increased peristaltic activity of the intestine, and a rapid expulsion of foeces, so that in a very short time the entire intestine was empty. A similar effect was produced by various vegetable purgatives, and by a solution of calomel in sodium bicarbonate. Atropin and calcium chloride seemed to have the opposite effect. The results of these experiments may be best seen in the following descriptions:

Pilocarpine hydrochlorate. Very dilute solutions of pilocarpine hydrochlorate produce evacuation of the foeces in *Sida* in 5 to 10 minutes. The rapidity with which the activity is produced varies with the concentration of the solution. Animals placed in 1% pilocarpine solution show immediately a marked activity of the intestine, as well as much increased movements of the post-abdomen. The peristaltic waves may be clearly observed, and, in addition, there are often seen sharp constrictions of the intestine. The expulsion of the foeces is very rapid and complete, so that at the end of a few minutes the intestine is entirely free from solid foeces, and is filled on the contrary with a clear greenish fluid. If the solution of pilocarpine be diluted ten times the action is still strong, but somewhat slower. Further dilution merely delays the action; and evacuation of the foeces was produced by a mixture of 1 c.c. 0.1% pilocarpine + 10 c.c. water, within 20 minutes.

The green fluid which fills the intestine after the expulsion of the solid foeces is derived mainly from the anterior portion of the gut; and it seems probable that the diverticula from the stomach play a rôle in its production. These diverticula are

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almost always filled with a similar fluid in normal animals, although it is possible that the green coloring matter is derived from the food. The increase in the fluid in the intestine is very noticeable after the foeces have been expelled by the action of pilocarpine; and although it is impossible in any way to measure the actual amount of fluid secreted before and after the administration of pilocarpine, there seems to be no doubt that the quantity in the intestine is much increased. In some cases after the foeces are discharged the intestine continues to empty out this green material, which after a time becomes replaced partly by a more colorless fluid. The rate of the heart beat does not seem to be noticeably increased by pilocarpine, and no conspicuous changes take place in the respiratory movements. It is also of some interest in this connection to note the nature of the circulation of the blood. No definite blood-vessels are present, and the blood circulates in fairly definite directions without being confined to capillaries or small channels. The blood corpuscles may be watched moving within wide limits. It is therefore difficult to imagine that the blood could reach any of the organs (*e.g.*, the cells which secrete the fluid of the gut) under a pressure which could be compared with the blood pressure of higher animals. The anterior portion of the alimentary canal, together with the diverticula, is simply bathed in a stream of blood which moves along a wide channel the size of whose lumen offers no resistance to the flow. It seems fair then to assume that the normal secretion of the alimentary canal in this animal is practically independent of blood pressure, and that the increased secretory activity produced by pilocarpine does not depend on an increase in blood-pressure, since the rate of the heart beat is not increased. Loeb[1] has already drawn attention to the fact that secretion occurs in organisms in which no circulatory system is present and in which blood pressure can therefore play no part.

An attempt was made[2] without satisfactory results in rabbits to determine whether or not calcium chloride is capable of inhibiting the activity of the intestine brought about by pilocarpine.

[1] J. LOEB: *Science*, 1898, N. S., Vol. VII, p. 154.

[2] J. B. MACCALLUM: *Archiv. f. d. ges. Physiologie*, 1904, Bd. 104, S. 421.

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This question is more easily tested in these crustaceans. Five specimens of *Sida crystallina* were placed in a mixture of 10 c.c. water + 1 c.c. 0.1% pilocarpine, which was found to be about the maximal dilution at which expulsion of the foeces could be brought about within a short period of time. Five similar specimens were placed at the same time in a mixture of 10 c.c. m/6 $CaCl_2$ + 1 c.c. 0.1% pilocarpine. A third group of five specimens was placed in pure m/6 $CaCl_2$. In the last solution there was no increase of peristaltic activity and no expulsion of foeces. In the first two solutions characteristic peristaltic activity of the intestine began about 10 minutes after the animals were placed in the solutions. The two groups of animals behaved in entirely the same way. Fifteen minutes after the beginning of the experiment the intestine of one of the animals in each group was empty. After 20 minutes, three of the first group, and four of the second group had expelled all their foeces. In 25 minutes all the animals in both groups had entirely emptied their intestines. Many similar experiments were made, and in no case was the action of pilocarpine at all delayed by the presence of calcium chloride.

In a mixture, however, of 10 c.c. 1% atropine sulphate + 1 cc. 0.1% pilocarpine, no evacuation of foeces whatever took place. Here the action of pilocarpine was evidently inhibited by the presence of a large proportion of atropin, a fact which is in accordance with what is known of the general actions of these two drugs in higher animals. In pure 1% atropine solution or in pure m/6 $CaCl_2$ solution there was no increase of intestinal activity, and no expulsion of foeces.

Barium chloride, sodium citrate, sulphate and fluoride. In solutions of any of these salts the intestine of *Sida crystallina* is quickly set into activity and the foeces are expelled. Stronger solutions must be used, however, than in the case of pilocarpine. In a mixture of 10 c.c. H_2O + 2 c.c. m/6 $BaCl_2$ the expulsion of foeces begins in 5 to 10 minutes, and at the end of 20 minutes the intestine is usually quite empty. Stronger solutions of $BaCl_2$ act more rapidly. The action is essentially similar to that described for pilocarpine.

A similar result is obtained more slowly in m/6 solutions of sodium citrate, sulphate, or fluoride. In all of these solutions

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also, and especially in $BaCl_2$, the secretion of fluid into the intestine seems to be increased.

Vegetable purgatives. The dried extracts of cascara sagrada and aloin are practically insoluble in distilled water. In a slightly alkaline solution however, as explained before[1], they become rapidly dissolved. The extract of aloin is more easily soluble in hot alkaline solutions. Solutions of these purgatives were made in m/24 $NaH\ CO_3$ solution.

In m/24 solution of sodium bicarbonate alone, the intestine of *Sida crystallina* remains for a considerable time unaffected. The animals often remain for an hour in this solution without expelling the foeces. Sometimes, on the other hand, this solution acts slowly and the foeces are after a considerable time partially cast out. The intestine is seldom completely emptied.

A saturated solution of cascara sagrada in m/24 $NaHCO_3$ begins to act very quickly, and active peristaltic waves appear in the intestine of the crustacean in a very few minutes. Usually within 10 minutes the intestine is entirely empty.

A similar solution of extract of aloin in m/24 $NaHCO_3$ acts somewhat more slowly than the cascara solution, but within half an hour the intestine is usually entirely empty.

Specimens of *Sida* placed in an aqueous solution of colchicin expel their foeces within 20 minutes.

Calomel. Calomel is practically insoluble in distilled water. On shaking it, however, in m/24 solution of $NaHCO_3$ the mixture gradually becomes gray. If this be filtered, the filtrate gives with H_2S a black precipitate indicating the presence of mercury. Specimens of *Sida* placed in this filtrate show very rapidly an increased activity of the intestine. Five of these animals were placed in the filtrate from a mixture of ½ g. calomel + 10 c.c. m/24 $NaHCO_3$. Five similar animals were at the same time placed in pure m/24 $NaHCO_3$. After they had been in the solutions 5 minutes the animals in the calomel filtrate showed active intestinal movements and the expulsion of foeces began. In the animals in $NaHCO_3$ no change was noticed. Nine minutes after the beginning of the experiment the intestines of two of the ani-

[1] J. B. MACCALLUM: *University of California Publications, Physiology,* 1904, Vol. I, p. 163; and *Archiv f. d. ges. Physiologie,* 1904, Bd. 104, S.421.

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mals in the calomel solution were empty. Eleven minutes after the beginning of the experiment four of these were empty, and twenty minutes after the animals were placed in solutions the intestines of all those in the calomel solution were quite empty. The animals in pure $NaHCO_3$ showed throughout this period no increased peristalsis and expelled no foeces.

Conclusions.

1.—Pilocarpine hydrochlorate in very dilute solutions causes in *Sida crystallina* active intestinal peristalsis, and complete evacuation of the foeces. This action of pilocarpine is not lessened by mixing $CaCl_2$ with it. Mixtures, however, of pilocarpine and atropin are relatively inactive.

2.—A similar activity of the intestine of *Sida* is produced by placing the animals in solutions of $BaCl_2$, sodium citrate, sulphate and fluoride. Solutions of the dried extracts cascara sagrada and aloin in m/24 $NaHCO_3$, as well as aqueous solutions of colchicin, also cause expulsion of foeces in *Sida* when the animals are placed in the solutions. The filtrate from a mixture of calomel in m/24 $NaHO_3$ has a similar effect. No such action is produced by pure m/24 $NaHCO_3$.

3.—An apparent increase in the secretion of fluid into the intestine of *Sida* takes place as a result of the action of these drugs. This is of interest in an animal which possesses no closed blood vascular system, and in which therefore blood-pressure cannot play a rôle in secretion.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 7, p. 71. February 2, 1905

ON THE VALIDITY OF PFLÜGER'S LAW FOR THE GALVANOTROPIC REACTIONS OF PARAMECIUM.

(Preliminary Communication.)

BY

FRANK W. BANCROFT.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

1. The response of Paramecium to most stimuli is preeminently a reversal of their normal unstimulated position, so that they strike forward.

2. When the constant current is made this reversed position of the cilia takes place only at the cathode end of the animal, and continues at that end during the flow of the current.

3. When a current of sufficient strength is broken, this reversed position of the cilia occurs only at the anode.

4. During the flow of the current the anode is in the unstimulated condition because a reversal of the anodal cilia can be brought about by chemical stimulation during the flow of the current.

5. If, for any reason, the cilia all over the body were already striking forward before the current was made, then the making of the current causes the anodal cilia to return to their normal unstimulated position, while the cathodal cilia remain in the stimulated condition.

6. All these facts are strictly in accordance with Pflüger's law, which is thus seen to hold for the reaction of Paramecium to the constant current, so far as the ciliary motions are concerned.

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PHYSIOLOGY

Vol. 2, No. 8, pp. 73–81. February 2, 1905

ON FERTILIZATION, ARTIFICIAL PARTHENOGENESIS, AND CYTOLYSIS OF THE SEA URCHIN EGG.[1]

BY

JACQUES LOEB.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

I.

In my earlier papers on artificial parthenogenesis I believe that I have shown it is generally possible to cause unfertilized eggs to develope to the larval state by means of certain physicochemical agents. The question whether the entrance of the spermatozoon into the egg brings about changes in the latter, similar to those which are caused by specific agents in artificial pathenogenesis, remained in large part unconsidered. So many facts concerning artificial pathenogenesis, however, have now been collected that the problem of the connection between artificial parthenogenesis and fertilization by sperm may be attacked.

As has been pointed out already, the eggs of different animals exhibit in different degrees the tendency toward parthenogenesis. The eggs of the starfish are, as I have already demonstrated in former years for Asterias[2], and as I have shown this year also for Asterina, in a condition to develope into normal larvae without any recognizable external interference. By artificial interference we only increase the number of the eggs that are capable of developing parthenogenetically in this group. The eggs of the sea-urchin, on the contrary, as shown by the researches carried on thus far at the Pacific Ocean, at the Atlantic

[1] Translated by John Bruce MacCallum from *Pflüger's Archiv.*, Bd. 103, S. 257, 1904.

[2] *Pflüger's Archiv.*, Bd. 99, S. 323, 1903.

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Ocean, and in Naples, possess only a slight tendency toward parthenogenetic development, and are never seen to develope without certain external interference.

The means by which the sea-urchin's eggs may be caused to develope into larvae are very limited, and I have found only a single method which leads to results, namely, the increasing of the osmotic pressure of the surrounding solution. It is simplest to take a relatively high concentration and leave the eggs in the solution for only a short time. If they be then replaced in normal sea-water they begin to develope. With the proper increase in osmotic pressure, and the appropriate time of exposure, the majority of the eggs develope into larvae.

I have further demonstrated by experiment that the transportation of the eggs from the hypertonic solution into normal sea-water is necessary only when the increase in the concentration of the sea-water is considerable.

As I had formerly observed[1], and as indeed is self-evident, an increase in the concentration of the sea-water above a certain limit exercises an inhibiting action on the segmentation and development of the sea-urchin egg. By careful experiment, however, it is possible to find an increase in concentration which is not high enough to suppress the development of the egg in the first twenty-four hours, and is still sufficient to produce artificial parthenogenesis in at least a small percentage of the eggs. In such hypertonic solutions I have seen unfertilized eggs of the sea-urchins develope into swimming blastulae without finding it necessary to remove the eggs from the solution and to transfer them to normal sea-water.[2] This fact is of theoretical importance because it shows that the loss of water on the part of the eggs is of greater significance for artificial parthenogenesis than the absorption of water when the egg is replaced in the normal sea-water.

That this conclusion is correct is further shown by the fact that it is not possible to produce a parthenogenetic development or segmentation by transferring unfertilized eggs from normal sea-water to diluted sea-water, and leaving them there either

[1] *Journal of Morphology,* Vol. 7, p. 253, 1892.

[2] *American Journal of Physiology,* Vol. 4, p. 178, 1900.

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permanently or temporarily. I have made many experiments of this nature, and all proved to be absolutely negative. If the entrance of water into the unfertilized egg were the essential condition for producing the development in artificial parthenogenesis, it would be possible to observe at least a beginning of the development when eggs are transferred from normal to diluted sea-water. This, however, as has been said, is not the case.

We thus come to the conclusion that the loss of water on the part of the egg is the essential factor in the artificial parthenogenesis, which is caused by increasing the concentration of the sea-water.

I have, up to the present time, found no other method by which it is possible to obtain swimming larvae from the eggs of the sea-urchin. If the eggs be placed for a long time in alkaline sea-water they begin to segment, but the eggs never develop into larvae when they are transferred again to normal sea-water. One may similarly observe the onset of development, *i.e.*, the first processes of segmentation, when one places the eggs temporarily in weakly acid sea-water. The statements of other authors that they have occasionally produced larvae from sea-urchin eggs by means of acids, have never convinced me, and I cannot avoid the suspicion that the authors in question have mistaken for sea-urchin larvae parasitic organisms which are found in the sea-urchin, and have reached the egg-culture from the circulatory system or from the intestine.

The possibility of causing the development of unfertilized eggs by the withdrawal of water seems to be rather generally distributed. In recent years I have succeeded in this way in producing[1] swimming larvae from the unfertilized eggs of various molluscs *(Lottia gigantia,* and different species of *Acmaea),* while Kostanecki[2] had already previously shown that in *Mactra* the first segmentation could be produced in this way.

Among Annelids, Fischer has produced artificial parthenogenesis in *Nereis limbata* by a temporary increase of the osmotic pressure of the sea-water[3]; and Bullot has made similar experi-

[1] *University of California Publications, Physiology,* Vol. 1, 1903.

[2] *Bulletin de l'Acad. des Sciences,* Krakau, 1902.

[3] FISCHER: *American Journal of Physiology,* Vol. 9, 1903.

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ments with a form of Ophelia.[1] Among vertebrates, Bataillon has caused, in the same way, a series of segmentation processes in the unfertilized egg of frogs and Petromyzon.[2]

It is, however, to be observed that although the osmotic method is in some forms the only, and in many the best, method by means of which artificial parthenogenesis can be brought about, there are nevertheless other efficacious methods which in certain forms give artificial parthenogenesis. There is no doubt that the addition of a small quantity of a suitable potassium salt to the sea-water causes the eggs of Chaetopterus to develope,[3] and that the same thing is accomplished in the case of Amphitrite[4] by calcium salts. But in both cases the development of the larvae is an abnormal one; the eggs develope into swimming larvae without *cell division*. I had already observed this, and Frank Lillie[5] has proven it beyond a doubt through cytological examination of the eggs. With the eggs of the starfish the temporary addition of a little acid to the sea-water leads to an increase in the number of eggs developing parthenogenetically.[6] But the development here is the result of nuclear and cell division, although occasionally the cell division may for some time not keep pace with the nuclear division.[7]

While the raising of the osmotic pressure of the sea-water is not the only method by which unfertilized eggs may be caused to develope into larvae, it is nevertheless the most general. It seems, further, that the development of the eggs brought about by the increasing of the concentration resembles more closely that which results from the entrance of spermatozoa. This leads to the idea that a wider analysis of the process of the osmotic fertilization will perhaps afford a wider agreement with the pro-

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cesses to be observed in normal fertilization. This idea gave the stimulus for the experiments presented below.

II.

The fertilization of the sea-urchin's egg by a spermatozoon is characterized by the fact that the egg forms a fertilization membrane. In the parthenogenetic development of the same egg brought about by the increasing of concentration of the sea-water no membrane formation takes place. This difference is so characteristic that it might lead to the opinion that the process of the osmotic fertilization has only little in common with the course of fertilization with sperm.

I have studied more carefully the course of the membrane formation in the sea-urchin's egg after the entrance of the spermatozoon. Under ordinary conditions the process goes forward suddenly. After one to three minutes following the addition of the sperm to the eggs one sees each egg become suddenly surrounded by a membrane. How the process of membrane formation goes on, and what the membrane itself is, may only be conjectured in such cases. I have, however, found that eggs of Strongylocentrotus which had lain in sea-water for 24 hours before the fertilization, formed the membrane more slowly, so that here one can follow the mechanism of the process. In such eggs the membrane frequently does not appear in all parts, but one sees that after the addition of the sperm there are formed, on the surfaces of the eggs, vesicles which afterward coalesce and then give rise to the fertilization membrane. The vesicle formation, however, consists of the extrusion (or the secretion under pressure?) of watery fluid by the protoplasm, by means

CORRECTION.

Page 76, seventh line from bottom, in footnote, read "inorganic acid," instead of "inorganic salt."

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The fact that the fluid is secreted under pressure accounts for the round, usually quite spherical, surface of the membrane. One of the essential actions of the fertilization by sperm consists in the expression of fluid from the egg.

This is, however, perhaps only one phase of the process. I have already stated, ten years ago, the fact that one action of the entrance of the spermatozoon into the sea-urchin's egg consists in the increasing of the power of swelling of the egg. I ascribed this process to an increased osmotic pressure in the egg caused by the entrance of the spermatozoon, and sought to place the process of egg fertilization on a parallel with that of muscle stimulation, since the latter results in the taking up of water by the muscle.[1] But I have arrived at the conception that the taking up and giving off of water in the animal organs does not depend entirely on osmotic pressure. Whatever the forces may be, however, upon which the interchange of fluid in the tissues depends, it is possible that in the fertilization the egg takes up fluid, and certainly it is possible that, following directly on this, there is an expression of water on the part of the egg.

It must now be remembered that the process of fertilization by sperm goes on very rapidly, while the production of artificial parthenogenesis is a very much slower process. The whole character of the membrane formation is only comprehensible when suddenly a very considerable secretion of water takes place at the boundary of the protoplasm and the superficial covering of the latter. In the ordinary experiments on artificial parthenogenesis of the sea-urchin's egg the osmotic pressure of the sea-water is increased only about 30-50%. It was therefore to be expected that with higher osmotic pressure the changes in the egg would proceed more violently, and that under these conditions also membrane formation would perhaps occur in unfertilized eggs. This suspicion was confirmed.

The experiments were carried on in concentrated solutions of salts and cane sugar.

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[1] LOEB, J.: *Pflüger's Archiv,* Bd. 55, S. 525, 1894, as well as Woods Hole Lectures, delivered 1893, Boston, Ginn & Co., 1894. Schücking, who lately has published observations on the taking up of water by the egg directly after the fertilization, seems to have overlooked my work on this subject. SCHUCKING, *Pflüger's Archiv,* Bd. 97, S. 58, 1903.

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If unfertilized eggs of *Strongylocentrotus purpuratus* be placed in a 2½ to 1½ n NaCl solution, there begins in a short time a membrane formation, which often, but not always follows a cytolysis of the egg. The membrane formation takes place in the same way as in the fertilization. Often the secretion of fluid takes place quite uniformly on the whole surface of the egg protoplasm, between this and the surface lamella which surrounds the unfertilized egg. In this case the membrane becomes suddenly raised up on all sides, as in the normal fertilization. In many cases, however, the process of membrane formation is here seen to go on in stages, *i.e.*, at certain parts of the surface a secretion takes place, so that the surface lamella at this place is raised up in the form of a small vesicle. The process then goes on in another place, until finally the surface lamella is everywhere elevated and the round, homogeneous, characteristic fertilization membrane is formed. Usually, however, the process does not stop here, but there occurs, after the membrane formation, a cytolysis of the egg, *i.e.*, the egg loses its pigment, and its structure, and becomes changed into an empty shadow. In so doing it increases largely in volume.

A concentrated cane sugar solution exerts also the same action. In 2½n and 2n cane sugar solution the same phenomena make their appearance.

It is scarcely necessary to remark that the conditions for this experiment may be varied in the most diverse ways. According to the concentration of the solution the phenomena occur more or less violently. The appearances also change with the nature of the solutions. Since, however, I intend undertaking a systematic study of the influence of the chemical nature of the substance upon the cytolysis, I shall not go into the subject more particularly at present.

The process of the membrane formation in the concentrated solutions proceeds in two phases: First, a shrinkage of the egg takes place, doubtless a result of loss of water; then a more or less distinct swelling of the shrunken egg occurs. That this swelling occurs without exception, however, I cannot state with certainty. After the shrinkage ceases, and after the beginning of the swelling, the membrane formation sets in. It appears as

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if the egg were seeking to rid itself, by secretion, of the water entering it during the swelling.

After the formation of the membrane, the cytolysis of the protoplasm of the egg follows almost without exception, often directly, many times after a longer interval. This process occurs usually, if not always, with a very great increase in the volume of the egg.

It is a striking phenomenon that in these experiments the cytolysis takes place mostly in those eggs which have formed a membrane. This led me to inquire whether fertilized eggs—which have formed a membrane under the influence of the spermatozoon—do not undergo cytolysis more quickly and in greater number than the unfertilized eggs. This is, in fact, the case. In order to show this one may use solutions which are almost or completely isotonic with sea-water. If freshly fertilized sea-urchin's eggs, for example, be placed in a ⅝ m. solution of Na_2SO_4, a large number, perhaps half of all the eggs, will be found to have undergone cytolysis after one and one-half hours. If unfertilized eggs be placed in the same solution, cytolysis will be found to have occurred in a much longer time (twenty-four hours) in a very small percentage of the eggs.

It will perhaps appear paradoxical to many that the eggs in a 2 m solution of a salt or of cane sugar begin, after an initial shrinking, to swell. This paradoxical behavior seems, however, to be very widespread. Five years ago I found that if a frog's muscle be placed in hypertonic NaCl solution, the muscle first loses in weight, then, however, gains; and this gain increases within certain limits with the concentration of the salt solution. While the frog's muscle, for example, gains only about 0.7% of its original weight in a 1.05% NaCl solution in twenty-four hours, it gains about 13% of its weight in a 1.75% solution, and in a 2.8% NaCl solution it gains about 23.8%.[1]

With *Infusoria* also one finds after an initial shrinking, a swelling, when they are placed in hypertonic solutions of certain concentration. In this way an increase in the size of the vacuoles takes place in many forms, and a formation of water-con-

[1] LOEB, J.: *Pflüger's Archiv.*, Bd. 75, S. 303, 1899.

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taining vesicles under the surface lamella,[1] which possibly is comparable with the formation of the fertilization membrane described here.

It is possible that the hypertonic salt or sugar solution makes the egg permeable for the outside solution, while certain constituents which produce an osmotic pressure are retained in the egg. This would necessitate a swelling of the egg after the initial shrinkage. Such an explanation would be in harmony with Starling's work on the formation of lymph.

After what has been said it will seem remarkable to no one that the eggs which were treated with a 2½ n NaCl or cane sugar solution were no longer in a condition to develope. If one wish to cause the unfertilized eggs to develope into larvae, one must use solutions of lower osmotic pressure, in which the eggs are not so greatly injured. Then no membrane formation takes place.

Though I am now of the opinion that one phase in the process of fertilization of the egg consists in the expulsion of fluid from the protoplasm by which the formation of the fertilization membrane is brought about, I still do not believe that this phenomenon of secretion necessarily proves that the spermatozoon alters to any extent the osmotic pressure of the egg. It is possible that the spermatozoon causes a taking up of fluid and a later secretion of fluid by other means.

[1] According to unpublished observations which Dr. Bullot has made in my laboratory.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 9, pp. 83–86. February 25, 1905

ON AN IMPROVED METHOD OF ARTIFICIAL PARTHENOGENESIS.

BY

JACQUES LOEB.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

In a series of papers I have described a method by which it is possible to produce swimming larvae from the unfertilized egg of the sea-urchin. This method consists in putting the sea-urchins' eggs for about two hours into sea-water whose concentration is raised by the addition of about 15 cc. 2½N NaCl to 100 cc. of sea-water. The development of these eggs, however, differs in four essential points from that of the eggs fertilized with sperm. In the first place, the eggs fertilized with sperm form a characteristic membrane as soon as the spermatozoon has entered, while the unfertilized eggs treated with hypertonic sea-water develop without the formation of a membrane. Second, the rate of development is considerably faster in the fertilized egg than in the egg caused to develop parthenogenetically. Third, the larvae originating from fertilized eggs rise to the surface of the water as soon as they begin to swim, while those originating by the above mentioned osmotic process swim at the bottom of the dish. Fourth, the number of larvae developing from fertilized eggs is, as a rule, practically a hundred percent., while in the case of artificial parthenogenesis a much smaller percentage of the eggs develop into swimming larvae. In the case of Arbacia I often succeeded in causing more than twenty per cent. of the unfertilized eggs to develop, but in the case of Strongylocen-

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trotus—the form of the sea-urchin common at Pacific Grove—I was rarely able to obtain even as high a per centage of developing eggs. Often enough only a fraction of one per cent. of the eggs yielded swimming larvae by the osmotic method of artificial parthenogenesis.

In thinking over the possible cause of this difference between the development of the egg fertilized by sperm and of the egg caused to develop by osmotic influences, it occurred to me that the spermatozoon might carry into the egg not one, but several, substances or conditions, each of which was responsible for only a part of the specific features of sexual fertilization; and that in order to completely imitate the action of the spermatozoon it might be necessary to combine two methods of artificial parthenogenesis, each of which alone imitated the process of sexual fertilization only partially. This latter idea proved correct far beyond my expectations.

In looking for substances which might be utilized for bringing about artificial parthenogenesis I tested the effects of ethylacetate, an ester, which had shown itself so effective in experiments on the control of the heliotropic reactions of fresh-water Crustaceans, recently published in this Bulletin. I found that if the unfertilized eggs of Strongylocentrotus are brought into 50 cc. of sea-water, to which about 0.6 cc. of a grammolecular solution of ethylacetate is added, and left in this water for from two to four minutes they not only form a membrane[1] when put back into normal sea-water, but begin also to segment. At a temperature of about 19° or 20° C. they begin to divide into two cells, about five or six hours after the treatment with ethylacetate, and later they may divide into more cells. In about twenty-four hours, however, all the eggs disintegrate, and I have never seen an egg which had been thus treated reach the blastula stage.

[1]The formation of a membrane in unfertilized eggs has already been observed by former experimenters. In 1887 O. and R. Hertwig noticed that the unfertilized eggs of a sea-urchin formed a membrane when put into sea-water to which chloroform had been added. Herbst noticed in 1893 that a number of substances, such as xylol, toluol, benzol, act in the same way. More recently he has obtained similar results with silver salts. (Herbst, Mittheilungen aus der zoologischen Station zu Neapel. Vol. 16, p. 445, 1904).

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If the eggs are left in the mixture of ethylacetate and sea-water they neither form a membrane nor do they segment.

On the other hand, the application of my old method of artificial parthenogenesis gave me this year poor results, owing possibly to the fact that the breeding season of Strongylocentrotus is just beginning. I succeeded in causing only a fraction of one per cent. of the unfertilized eggs treated with hypertonic sea-water (100 cc. sea-water plus 15 cc. 2½N NaCl) to develop into swimming larvae: and even these larvae, as a rule, died in an early larval period.

When I applied both methods in succession, however—namely, the original osmotic method and the treatment with ethylacetate, I obtained surprising results. Instead of a fraction of one per cent. of the eggs developing, I had it in my power to cause ninety to one hundred per cent. of the eggs to develop. All the eggs formed the membrane which is characteristic of the egg fertilized with sperm. The rate of segmentation was practically the same as that of the eggs of the same female, fertilized with sperm. A large percentage of the blastulae originating from this combination of methods looked perfectly normal, and rose to the surface of the sea-water. Their further development into gastrulae and plutei occurred with the same velocity as that of the control eggs, which had been fertilized by sperm; and the larvae showed an equal degree of vitality.

The order in which these two methods, namely, that of treating the eggs with ethylacetate and with hypertonic sea-water, were applied was not immaterial. The results were much better when the eggs were first submitted to the hypertonic sea-water, and afterward to the ethylacetate, than if the order was reversed. The usual procedure was to put the unfertilized eggs for about two hours and twenty minutes into a mixture of 100 cc. sea-water and 15 cc. N NaCl at a temperature of about 19° or 20° C. The eggs were then put into normal sea-water, where they were left for about five or ten minutes and then put into another dish with 50 cc. sea-water. Then 0.6 cc. of a grammolecular solution of ethylacetate were added in drops and the whole stirred with a pipette, so as to bring about a rapid and thorough mixture of the ethylacetate and the sea-water, and to expose the eggs to the diluted ethylacetate.

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From time to time a few drops containing eggs were put into a watch glass containing about 10 cc. of pure sea-water, in order to ascertain whether the eggs were ready to throw out the membrane. As soon as the eggs formed the membrane when put into normal sea-water, they were transferred from the ethylacetate solution into normal sea-water. This was usually the case in from two to four minutes after the eggs had been put into the ethylacetate solution.

When the eggs were taken out a little too early,—namely, at a time when only a number, but not all, of the eggs formed a membrane,—it was found as a rule that only such eggs showed a normal and rapid segmentation as had formed a membrane. The other eggs, as a rule, did not segment at all, or if they segmented, which occurred only exceptionally, they segmented at the slow rate of the eggs treated only with hypertonic sea-water. The fact that in such cases only those eggs developed which had formed a membrane is another coincidence between this new method of artificial parthenogenesis and sexual fertilization.

It is thus obvious that we are now able to imitate the process of sexual fertilization in the egg of the sea-urchin completely and in all its essential features by purely physical and chemical means. The fact that a number of the parthenogenetic larvae raised by the new method seem to have the same vitality as the larvae produced by normal fertilization arouses the hope that it will now be possible to undertake the solution of the various problems for which the raising of parthenogenetic larvae in large numbers is a prerequisite.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 10, pp. 87-88. March 16, 1905

ON THE DIURETIC ACTION OF CERTAIN HAEMOLYTICS, AND THE ACTION OF CALCIUM IN SUPPRESSING HAEMOGLOBINURIA

(Preliminary Communication.)

BY

JOHN BRUCE MACCALLUM.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

1. Quillain (extract of Quillaja bark) and saponin, which are well known to possess a powerful haemolytic action, I have found to act also as diuretics in rabbits. If the flow of urine be increased by the injection of m/6 NaCl solution into the blood at a uniform rate, until the quantity of fluid secreted becomes constant, the injection of very minute quantities of quillain or saponin increases the flow of urine markedly.

2. This increased flow of urine may be suppressed by the injection of m/6 $CaCl_2$.

3. Quillain and saponin produce well marked haemoglobinuria, which may be partially or wholly prevented by the simultaneous injection of m/6 $CaCl_2$. If 2 c.c. 1% quillain (dissolved in m/6 NaCl) be diluted with 6 c.c. m/6 NaCl, and 2 c.c. of this mixture be injected into the blood vessels of a rabbit weighing 1,200 g., a marked haemoglobinuria will be produced. If the same amount of quillain be diluted with 6 c.c. m/6 or m/9 $CaCl_2$, and 2 c.c. of this mixture injected in the same way, there will be either no haemoglobinuria or a much slighter haemoglobinuria than in the former case.

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4. Defibrinated rabbit's blood is rapidly laked by very dilute solutions of quillain, saponin or digitalin. This process is influenced by certain salts, especially calcium and magnesium chloride. If a mixture be made of 1 c.c. blood + 5 c.c. m/6 NaCl + 3 drops 0.5% saponin, laking takes place partially in 3 minutes. and completely in 10 minutes; 1 c.c. blood + 5 c.c. m/6 or m/9 $CaCl_2$ + 3 drops 0.5% saponin, undergoes practically no change in 1 hour; 1 c.c. blood + 5 c.c. m/6 or m/9 $MgCl_2$ + 3 drops 0.5% saponin shows no change after 1 hour. If the NaCl solution, however, be replaced by m/6 KCl, Na_2SO_4 or sodium citrate, laking takes place somewhat more rapidly; m/6 solutions of cane sugar, Na_3PO_4, NaF, $NaHCO_3$ act in the same way as m/6 NaCl. $BaCl_2$, on the other hand, delays the haemolysis, as do $CaCl_2$ and $MgCl_2$. The same results were obtained with quillain and digitalin.

5. It is thus seen that these substances which are powerful haemolytics also act as diuretics; and $CaCl_2$ and $MgCl_2$, which inhibit the secretion of urine, also to some extent inhibit the haemolytic action. It seems possible that the same process which renders the red-blood corpuscles more permeable to the outward passage of haemoglobin, also renders the kidney cells more permeable for the fluid which passes through them; and it is possible that digitalin, quillain and saponin owe their diuretic action largely to their increasing the permeability of the kidney cells, especially those of the Malpighian corpuscles.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 11, pp. 89–92. March 16, 1905

ON AN IMPROVED METHOD OF ARTIFICIAL PARTHENOGENESIS.

(Second Communication.)

BY

JACQUES LOEB.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

In a previous note I stated that the process of fertilization and the subsequent development of the eggs of the sea-urchin could be repeated in all of its essential details by a combination of two methods of treatment, namely, by first putting the eggs for about two hours into hypertonic sea-water, and then by submitting them for a few minutes to sea-water to which a small quanty of ethylacetate had been added. It seemed desirable to ascertain which general group of chemical compounds was able to act like ethylacetate in the above experiment. Ethylacetate dissociates into ethylalcohol and acetic acid. The solution of ethylacetate which I used in this experiment was prepared about six months ago, and contained a considerable amount of free acetic acid. I tried whether it was possible to substitute acetic acid for the ethylacetate. It was found that if the unfertilized eggs of the sea-urchin were put at a temperature of about 20° from one to two minutes into 50 c.c. of sea-water to which about 4 c.c. of N/10 acetic acid were then added, the eggs formed the membrane characteristic of the process of fertilization *after they were put back into normal sea-water.* When the eggs were left in the acidulated sea-water they formed no membrane, nor did they form a membrane when they were taken out of the acidulated sea-water a little too early or too late. When a smaller amount of acetic acid than that mentioned above was added, the time

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of exposure of the eggs had to be increased; and when a larger amount was used it had to be shortened. In regard to the process of membrane formation, the acetic acid therefore behaved like the ethylacetate.

The acetic acid acted also much like the ethylacetate in regard to the effect upon the subsequent development of the unfertilized egg. Unfertilized eggs that had been exposed from one to two minutes to a mixture of 50 c.c. sea-water + 4 c.c. N/10 acetic acid disintegrated in less than twenty-four hours without developing; but when the eggs before being put from one to two minutes into the mixture of sea-water and acetic acid were kept for about two hours in a mixture of 100 c.c. of sea-water + 14 or 15 c.c. of 2½ N NaCl, they not only formed a membrane after having been exposed to the acid, and transferred back into normal sea-water, but they also segmented and developed into larvæ. When the time of exposure was properly chosen they developed at the same rate and in exactly the same way as the eggs fertilized with sperm. The larvæ rose to the surface like the larvæ produced from the eggs fertilized by sperm.

In these experiments the time of exposure of the egg to the acid is the most important factor. I gained the impression that a comparatively short exposure of the eggs to a high concentration of acid gave better larvæ than a longer exposure to a lower concentration of the acetic acid in sea-water; and for this reason the above-named proportions of sea-water and acetic acid were chosen. When 5 or 6 c.c. of acid were added to 50 c.c. of sea-water the time of effective exposure became so short that it was difficult to remove the eggs fast enough from the solutions. The character of the larvæ developing from the unfertilized eggs depends upon the time of exposure of the eggs to the acid. As a rule I proceeded in the following way: The eggs were transferred from the hypertonic sea-water to normal sea-water and than 4 c.c. of N/10 acetic acid were added under constant squirting of the sea-water with a pipette. This procedure occupied about fifteen seconds. Then, at intervals of fifteen seconds, lots of eggs were taken out and put back each into a separate dish containing normal sea-water. The first and second lot thus taken out formed as a rule no membrane. The membrane formation began with a

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few eggs in the third, and with more in the fourth, and became general with the later lots until after about three minutes, when it again ceased. Sometimes the membrane formation did not begin in the third, but in a later lot. I found that only such eggs developed as had formed a membrane; and of the eggs that formed a membrane, chiefly those yielded perfect larvæ (which rose to the surface) that were contained in the first two or three lots in which the membrane formation first occurred. It is therefore obvious that for the purpose of producing normal larvæ from unfertilized eggs which have previously been treated with hypertonic sea-water, the time of exposure of the eggs to the acid may exceed but slightly the minimum required to call forth the process of membrane formation. It must possibly exceed this time by a few seconds, since I have often observed that in the first lot in which the membrane formation began not every egg which formed a membrane developed immediately, or at all. In the later lots all the eggs form a membrane, and all may develop, but not all produce larvæ which rise to the surface; it seems that the percentage of developing eggs that give rise to such larvæ is greatest in the first two or three lots in which the membrane formation begins.

A number of other acids were then tried. The effects of acetic acid on membrane formation could be easily repeated with formic acid and CO_2; 4 c.c. of N/10 formic acid were about as effective as 4 c.c. of N/10 acetic acid. I applied CO_2 in the form of carbonated water (Shasta water) which was rendered isotonic with sea-water through the addition of a sufficient quantity of a 2½N NaCl solution. About 60 c.c. of Shasta water had to be added to 50 c.c. of sea-water to produce the same results as with 4 c.c. N/10 acetic acid.

Experiments with HCl have given thus far practically negative results, and those with tartaric and oxalic acid, absolutely negative results. When unfertilized eggs were put for about one to two minutes into 50 c.c. of sea-water to which 4 c.c. N/10 HCl were added, a few eggs formed a membrane, when taken out of the solution and put back into normal sea-water; but while the membranes formed after the treatment with acetic, formic, and carbonic acid were as perfect as those formed through fertiliza-

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tion with sperm, those formed by the treatment with hydrochloric acid were abnormal. It is, however, possible that a continuation of these experiments with hydrochloric, tartaric, and oxalic acids may lead to better results.

The results obtained with acetic acid gave rise to the question whether the action of ethylacetate was not partially or entirely due to the free acetic acid. When the ethylacetate was titrated for acid it was found that it contained enough free acetic acid to account for its effect. A fresh solution of ethylacetate which contained only a trace af acetic acid did not call forth the process of membrane formation. Ethylformiate which had a slightly acid reaction called forth the formation of the membrane, if applied in the way described for ethylacetate.

In my first note I stated that the spermatozoon might possibly carry two different agencies into the egg, one of which acted like the ethylacetate, while the other acted similarly to the hypertonic sea-water. It seems certain that the action of the acid does not consist in a merely quantitative increase of the effects of the hypertonic sea-water. If we treat the unfertilized eggs with acid alone (without submitting them to the hypertonic sea-water) every egg which forms a membrane disintegrates in less than twenty-four hours. The eggs which are treated only with hypertonic sea-water for two hours form no membrane, and do not disintegrate. This indicates a more than merely quantitative difference in the effects of the two agencies. The combination of both methods leads, if the proper attention is given to temperature, concentration, and time of exposure, to a process of development which to all appearances is identical with the effects of the entrance of a spermatozoon.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 12, pp. 93-113 May 16, 1905

THE DIURETIC ACTION OF CERTAIN HAEMOLYTICS AND THE INFLUENCE OF CALCIUM AND MAGNESIUM IN SUPPRESSING THE HAEMOLYSIS.

(Second Communication.)

BY

JOHN BRUCE MACCALLUM.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

The following is a description of a number of experiments with haemolytics, to determine, in the first place, their possible influence on the secretory activity of the kidney; and, in the second place, whether or not those substances which inhibit renal secretion also inhibit haemolysis.

Methods.

In all of the experiments rabbits were used weighing from 1,200 to 1,500 g. Morphine was employed as an anaesthetic, 4-5 c.c. 1% solution of the hydrochlorate being administered subcutaneously in each case. The urine was collected by inserting a cannula directly into the bladder. The abdominal cavity was opened sufficiently to allow the bladder to protrude, and by means of a purse-string suture the cannula could be firmly tied in, and all the urine secreted in a unit of time could be readily measured. The animals were protected as far as possible from loss of heat.

[1]A preliminary report of these experiments appeared in the University of California Publications, Physiology, Vol. II, March 16, 1905.

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In order to test the action of the various substances on the flow of urine, m/6 NaCl solution was injected at a constant rate (2 c.c. per minute) into the marginal vein of the ear. This was accomplished by an ordinary injection apparatus with a pressure bottle connected with the water tap. The salt solution passed from a second bottle into a long coil of rubber tubing, which was immersed in water kept constantly at 40° C. In the end of this tubing was fastened a hypodermic needle, which was inserted into a vein of the rabbit's ear. A short time after the beginning of the injection the flow of urine began, and gradually increased until it reached a constant. The substance to be tested was then injected into the vein of the other ear, or along with the NaCl solution. All the solutions were made as nearly as possible isosmotic with the rabbit's blood. The saponin, digitalin, etc., were dissolved in m/6 NaCl solution.

In testing the influence of the salts on haemolysis the blood was defibrinated in the ordinary way and 1 c.c. in each case mixed with equal quantities of NaCl and of the various salt solutions to be tested. The same number of drops of the haemolytic were then added to each mixture and the time of laking determined. In this way the mixture containing NaCl was taken as a normal, and the effects of the other salts compared with this. In measuring the haemolytic solutions by drops a number of pipettes of the same size were used. From each of these pipettes 15 drops of the solution measured always 1 c.c.

The saponin and digitalin used were the pure powdered preparation made by Merck. The quillain was a dried extract of Quillaja bark.

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Diuretic Action of Saponin and Quillain.

The influence of saponin on the flow of urine may be observed in the following experiment:

TABLE I.

Time.	m/6 NaCl injected intravenously.	Urine.
10:15		
10:20	10	2
10:25	20	4
10:30	20	6
10:35	10	7.5
10:40	10	8.0
10:45	10	8.2
10:50	10	7.9
10.56	Injected 2 c.c. 1/20% saponin in m/6 NaCl.	
11:00	10	10.5
11:05	10	11.0
11:10	10	11.0
11:15	10	11.0
11.16	Injected 1 c.c. 1/20% saponin.	
11:20	10	12.2
11:25	10	13.2
11:30	10	12.0
11:35	10	12.5

In the experiment no haemoglobin appeared in the urine. The The quantity of saponin injected was sufficient to produce a diuretic action, but the amount of haemolysis which it occasioned did not liberate haemoglobin in large enough quantities to be secreted in the urine. A large amount of saponin produces marked haemoglobinuria.

The diuretic effect of quillain may be seen in the following Table II. Here well marked haemoglobinuria results. The urine becomes deeply stained, and the characteristic absorption bands of oxyhaemoglobin can be readily made out with the spectroscope. This experiment shows also the action of $CaCl_2$ in suppressing the diuretic effect of the quillain, although it does not stop the secretion of haemoglobin after the latter has once been liberated from the red blood corpuscles. It was shown in a previous paper[1] that $CaCl_2$ has in general an antidiuretic effect, especially counteracting the diuretic effects produced by sodium salts. It was also pointed out in this paper that the production and suppression of diuresis was entirely analogous to the production and suppression of muscular twitchings in voluntary muscle, as described by Loeb.[2]

[1]J. B. MacCallum. Journal of Experimental Zoology, Vol. 1, No. 1, 1904.
[2]J. Loeb. Festschrift f. Fick, 1899; Archiv. für die gesammte Physiologie, XCI, p. 248, 1902.

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TABLE. II.

Time.	m/6 NaCl.		Urine.
10:40			
11:00	30		1.5
11:05	10		2.8
11:10	10		6.3
11:15	10		8.0
11:20	10		8.5
11:25	10		8.5
11:28	Injected 2 c.c. 1/4% quillain intravenously.		
11:30	10		8.0
11:35	10		11.0
11:40	10	haemoglobinuria	12.5
11:45	10	haemoglobinuria	12.0
11:46	Injected 5 c.c. m/6 $CaCl_2$ intravenously.		
11:50	10	haemoglobinuria	3.2
11:55	10	haemoglobinuria	4.0
12:00	10	haemoglobinuria	4.0

In this case the haemoglobinuria was very marked, and appeared about five minutes after the injection of the quillain. A number of experiments similar to this showed that in a rabbit which weighed 1,200 g. this dose of quillain always produces haemoglobinuria. The subsequent injection of $CaCl_2$ diminishes the flow of urine, but does not stop the haemoglobinuria. The secretion of haemoglobin by the kidneys is not stopped by $CaCl_2$ after it has once been liberated in the blood.

The following Table III shows an experiment in which the same quantity of quillain was given, but in this case it was diluted with m/6 $CaCl_2$ instead of with m/6 NaCl as in Table II. In other words, 2 c.c. of a mixture of 2 c.c. 1% quillain + 6 c.c. m/6 $CaCl_2$ were injected, while in the experiment of Table II there were injected 2 c.c. of a mixture of 2 c.c. 1% quillain + 6 c.c. m/6 NaCl. Practically no haemoglobin appeared in the urine when the mixture containing $CaCl_2$ was injected. The diuretic effect was also counteracted.

TABLE III.

Time.	m/6 NaCl.		Urine.
9:40			
9:45	30		1
9:55	20		4
10:00	10		5.8
10:05	10		8.3
10:10	10		8.5
10:12	Injected 2 c.c. { 2 c.c. 1% quillain, 6 c.c. m/6 $CaCl^2$		
10:15	10	No haemoglobinuria	6.2
10:20	10	No haemoglobinuria	4.6
10:25	10	No haemoglobinuria	7.0
10:30	10	No haemoglobinuria	4.2
10:35	10	No haemoglobinuria	6.0
10:40	10	No haemoglobinuria	6.0

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In other experiments, made as repetitions of this one, the same results were obtained; in one or two, however, there appeared in the urine 10 or 15 minutes after the injection of the quillain mixture a very slight trace of haemoglobin, not to be recognized certainly with the naked eye, but sufficient for identification with the spectroscope. This, however, was always in marked contrast to the deeply stained urine obtained by the injection of quillain + NaCl.

In some instances the $CaCl_2$ seemed to prevent the haemolytic action of the quillain without entirely inhibiting its diuretic influence. This is shown in Table IV. No haemoglobin whatever appeared in the urine, while the quantity of urine was at the same time considerably increased. The subsequent addition of a small quantity of quillain diluted with m/6 NaCl, instead of m/6 $CaCl_2$, immediately produced very marked haemoglobinuria.

TABLE IV.

Time.	m/6 NaCl.		Urine.
10:05			
10:20	40		0.5
10:25	10		1.0
10:30	10		1.0
10:35	10		3.4
10:40	10		4.0
10:45	10		3.5
10:50	10		3.5
10:55	10		3.5
10:56	Injected 2 c.c.	2 cc. 1% quillain 6 cc. m/6 $CaCl_2$	
11:00	10	No haemoglobinuria	4.6
11:05	10	No haemoglobinuria	4.6
11:10	10	No haemoglobinuria	9.0
11:15	10	No haemoglobinuria	13.0
11:20	10	No haemoglobinuria	10.0
11:25	10	No haemoglobinuria	9.0
11:30	10	No haemoglobinuria	10.0
11.31	1½ c.c. ¼% quillain in m/6 NaCl.		
11:35	10	Marked haemoglobinuria	6.0

The possibility suggests itself that the diuretic effect of the quillain, saponin, etc., might in part be due to the haemoglobin liberated in the blood. This, however, is not the case. Solutions of haemoglobin were prepared by isolating the blood corpuscles of rabbit's blood from the serum and treating them with about three times their volume of distilled water. This gave a clear solution containing oxyhaemoglobin and a very small quantity of rabbit's serum. A rabbit was then prepared as described above

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and a constant flow of urine established. Into the veins 2 c.c. of the haemoglobin solution were injected. Within ten minutes oxyhaemoglobin appeared in the urine, but the quantity of urine secreted in the units of time was not at all increased. The secretion of haemoglobin persisted for nearly an hour and then gradually ceased, the urine again becoming colorless. The rate of flow of urine, however, remained practically constant, and even showed a tendency to decrease. In this connection also it is again to be noted that a diuretic effect may be obtained by a quantity of saponin which is insufficient to produce haemoglobinuria.

Barium chloride seemed to have in this respect an action similar to that of $CaCl_2$: 2 c.c. of a mixture of 2 c.c. 1% quillain + 1 c.c. m/6 $BaCl_2$ + 5 c.c. m/6 NaCl produced only the slightest trace of haemoglobin in the urine. It has been shown before[1] that the effect of $BaCl_2$ on the muscles and glands of the intestine is quite the opposite to that of calcium chloride. Loeb[2] has, however, shown that with respect to the segmentation of the eggs of Fundulus $CaCl_2$ and $BaCl_2$ behave similarly in their antitoxic action toward sodium salts.

It was found that atropin exerted no influence on the production of haemoglobinuria by quillain. A mixture of 2 c.c. 1% quillain + 6 c.c. 1/10% atropin (both dissolved in m/6 NaCl) was as effective in producing haemoglobinuria as quillain + NaCl alone.

Digitalin was found to produce haemoglobinuria in rabbits in extremely small doses. While its diuretic action is well known, this action did not in these experiments seem to be nearly so powerful as that of saponin or quillain. At the same time, I did not succeed by the administration of $CaCl_2$ in obtaining any inhibition of the haemoglobinuria produced by digitalin. This may have been due to a failure in obtaining the minimal dose of digitalin, for, as shown below, the haemolytic action of digitalin is inhibited by $CaCl_2$ in a way quite similar to the inhibition of the action of saponin and quillain. It was found in many of the experiments that immediately after the injection of digitalin

[1]J. B. MacCallum, Am. Jour. of Physiology, Vol. 10, p. 101, 1903; p. 259, 1904; Pflüger's Archiv., Bd. 104, 1904.

[2]Pflüger's Archiv, Vol. 88, 1901.

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the urine came to an abrupt standstill, and did not flow again for five or more minutes. It then continued, and usually increased in quantity. This cessation of the flow of urine is similar to that obtained with $BaCl_2$ as previously described, and also to that which occurs after the injection of pilocarpine.

Influence of Calcium and Magnesium on Haemolysis.

Assuming that the haemoglobinuria in the experiments described above is a secondary phenomenon, depending on the production of haemolysis, I have made a large number of experiments with the object of comparing the influences of various salts on the haemolytic action of saponin, quillain, and digitalin. Pohl[1] found that acid sodium phosphate exerts a powerful protecting influence against the haemolytic action of solanin. He afterward observed that acid sodium sulphate has the same action. Acid sodium phosphate, according to Pohl, does not inhibit the haemolytic action of saponin. It does, however, protect against eel serum (ichthyotoxin). Nolf[2] has shown that the haemolytic action of ammonium chloride, and also of certain serums, is delayed by calcium acetate, barium chloride, and magnesium sulphate. The haemolysis caused by ox bile, however, took place more readily in the presence of these salts than in the presence of NaCl or KNO_3.

Hedon[3] states that acid sodium phosphate inhibits only to a limited extent the haemolytic action of solanin. All substances with an acid reaction to some degree retard haemolytic action, while substances possessing an alkaline reaction tend to hasten the action. Bashford[4] claims that only free solanin is active, and that acids prevent its separation. According to Markl,[5] NaCl exerts an inhibitory action on haemolysis.

In the following experiments the various tests were made under the same conditions of temperature, osmotic pressure, and concentration. The rabbit's blood was defibrinated and placed

[1]Pohl. Arch. International de Pharmacodyn. et de Therapie. VII, 1900.
[2]Annals de l'Institut Pasteur. 14, 1900.
[3]Arch. internat. de Pharmacodyn. et de Therapie. IX, 1902.
[4]Arch. internat. de Pharmacodyn. et de Therapie. VIII, 1901.
[5]Zeitsch. f. Hygiene. 39, 1902, S. 86.

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in a burette from which it could be accurately measured. No attempt was made to determine more than the comparative action of the various salt solutions. Since it was necessary in adding each salt solution, to considerably dilute the blood, it seemed impossible to place undiluted blood under the same conditions. Blood diluted with m/6 NaCl was therefore taken as a normal, and blood diluted with the same proportion of various other solutions compared with this, the same amount of the haemolytic being added in each case. This may be illustrated by the following Table V.

Time	1 c.c. blood 5 c.c. m/6 NaCl 3 drops 0.5% saponin	1 c.c. blood 5 c.c. m/6 $MgCl_2$ 3 drops 0.5% saponin	1 c.c. blood 5 c.c. m/6 $CaCl_2$ 3 drops 0.5 % saponin
A.M. 10:22			
10:24	change of color	no change	no change
10:27	almost transparent	" "	" "
10:30	laking almost complete	" "	" "
10:45	laking complete	" "	" "
P.M. 1:00	" "	corpuscles settled to bottom; supernatant fluid colored; mixture quite opaque on shaking.	same as $MgCl_2$ mixture

As shown in this table, $CaCl_2$ and $MgCl_2$ inhibit very markedly the haemolytic action of saponin. Whereas, a distinct change takes place in the tube containing NaCl in 4-5 minutes, no change is observable in either of the others for some hours. The former mixture becomes quite transparent; the mixtures containing $CaCl_2$ and $MgCl_2$ remain always opaque. That a slight degree of haemolysis has taken places in the latter mixtures after several hours is shown by the red coloraton of the fluid which lies above the corpuscles after these have sunk to the bottom of the tube.

Slight differences in the osmotic pressure of the solutions have no influence on this action. Thus, since it is difficult to obtain solutions of NaCl and $CaCl_2$, for example, which are exactly isosmotic, various concentrations were tested. The blood in one

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tube was diluted with 5 c.c. m/6 NaCl; in other tubes mixtures were made of blood with 5 c.c. m/6 $CaCl_2$, m/8 $CaCl_2$, and m/9 $CaCl_2$, respectively. In all the tubes containing $CaCl_2$ the haemolysis was inhibited, while that containing NaCl showed rapid laking on addition of the haemolytic.

In the same way the comparative effects of NaH_2PO_4, $CaCl_2$, and NaCl were tested, as shown in the following Table VI.

TABLE VI.

Time	1 c.c. blood 5 c.c. m/6 NaH_2PO_4 4 drops 0.5% saponin	1 c.c. blood 5 c.c. m/6 $CaCl_2$ 4 drops 0.5% saponin	1 c.c. blood 5 c.c. m/6 NaCl 4 drops 0.5% saponin
A.M. 11:26			
11:27	change in color	no change	change in color
11:30	partially laked	" "	partially laked
11:32	almost transparent	" "	almost transparent
11:40	laking complete	" "	laking complete
P.M. 2:30	" "	corpuscles sunk to bottom; supernatant fluid colored; quite opaque on shaking.	

TABLE VII.

Time	1 c.c. blood 5 c.c. m/6 NaH_2PO_4 3 drops 1% digitalin	1 c.c. blood 5 c.c. m/6 $CaCl_2$ 3 drops 1% digitalin	1 c.c. blood 5 c.c. m/6 NaCl 3 drops 1% digitalin
A.M. 11:39			
11:40	change in color	no change	change in color
11:43	partially laked	" "	partially laked
11:50	completely laked	" "	completely laked

With regard to the haemolytic action of saponin and of digitalin, acid sodium phosphate seems to behave like NaCl. There is no evident inhibitory action whatever. $CaCl_2$, on the other hand, exerts a very marked protective action in each case. The same results were obtained with quillain.

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This action of $CaCl_2$ is conditioned, however, by the concentration of the haemolytic. The striking effects described in the experiments outlined above were obtained with a quantity of the haemolytic which was in the neighborhood of the minimal quantity capable of haemolysing the amount of blood used. If more of the haemolytic be added the haemolysis occurs not only in the mixtures containing NaCl and NaH_2PO_4, but also in those containing $CaCl_2$ and $MgCl_2$. The rapidity of this action varies roughly with the concentration of the haemolytic, as shown in the following Table VIII.

TABLE VIII.

Time	1 c.c. blood 5 c.c. m/6 $CaCl_2$ 3 drops 0.5% saponin	1 c.c. blood 5 c.c. m/6 $CaCl_2$ 6 drops 05.% saponin	1 c.c. blood 5 c.c. m/6 $CaCl_2$ 8 drops 05% saponin	1 c.c. blood 5 c.c. m/6 $CaCl_2$ 10 drops 05% saponin
A.M. 11:04				
11:06	no change	no change	slight change in color	distinct change
11:07	" "	" "	partially laked	almost transparent
11:10	" "	slight change in color	almost completely laked	laking complete
11:20	" "	partially laked	laking complete	" "
11:30	" "	not complete	" "	" "
P.M. 1:00	" "	" "	" "	" "

Many other salts were tested in the same way, and in each case compared with NaCl and $CaCl_2$. It was found that $BaCl_2$ acted to some extent like $CaCl_2$ and $MgCl_2$, but inhibited the haemolysis much less powerfully. Sodium citrate, sodium sulphate, and potassium chloride seemed to slightly increase the rapidity of haemolysis. This, however, was not a conspicuous increase, and their influence seemed to be inconsiderable. Solutions of cane sugar, Na_3Po_4, NaF, and NaH CO_3 behave in the same way as NaCl. If mixtures are made as shown above, each containing the same quantity of blood and the same quantity of equimolecular solutions of these salts, the laking will take place on the addition of the same quantity of the haemolytic, approximately simultaneously in all cases.

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Pilocarpine hastens to some extent the haemolysis caused by saponin, digitalin, etc., and even alone it has a slight haemolytic action; 1 c.c. blood + 5 drops of 1% pilocarpine hydrochlorate became rapidly laked. With 8 drops of pilocarpine the laking took place in ½-1 minute. This was not due to the low osmotic pressure of the pilocarpine solution, because an equal quantity of distilled water added to the blood failed to cause laking in the same time.

Atropin was found to have no appreciable effect on the rapidity of haemolysis.

It is possible that it is by no means a coincidence that these substances which are powerful haemolytics act also as diuretics: and that $CaCl_2$ and $MgCl_2$, which inhibit the secretion of urine, also to some extent inhibit the haemolytic action. It is difficult to say by what process the haemoglobin is liberated from the red blood corpuscle, as indeed it is difficult to state definitely how fluid passes from the blood into the urine. It is sufficient to call attention to the fact shown by the above experiments, that the liberation of haemoglobin and the flow of urine may be to some extent controlled by the same conditions. If the haemolytics such as saponin cause haemolysis by increasing the permeability of the membranes of the red blood corpuscles, it seems possible that the diuretic effect of these substances may be due to a similar process in the kidney. If this be true, changes in permeability must play an important part in the action of these diuretics. And it is not impossible that the inhibition of the haemolytic action of saponin, etc., as well as the inhibition of the flow of urine by $CaCl_2$ and $MgCl_2$, may be due to a decreased permeability of the red blood corpuscle on the one hand, and of the kidney cells on the other.

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PHYSIOLOGY

Vol. 2, No. 13, pp. 105–112 May 16, 1905

THE ACTION OF PILOCARPINE AND ATROPIN ON THE FLOW OF URINE.

BY

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Although the actions of pilocarpine and atropin on the salivary glands, the pancreas, and the glands of the skin are very definite and well known, there seems to exist some doubt as to their action on the secretion of urine. W. H. Thompson[1] found that atropin diminishes the secretion of urine, although the blood pressure is at the same time slightly increased. Similar results were obtained by Walti.[2] Lazzaro and Pitini[3] found that in dogs pilocarpine causes a diminution of diuresis. According to Schmiedeberg,[4] the secretion of urine is not directly affected by pilocarpine. Cushny[5] also states that it is affected only by the loss of fluid through other glands.

The following is a description of a number of experiments undertaken with the object of arriving at a more definite conclusion with regard to the actions of these substances. In general it was found that when the flow of urine had been greatly increased by the injection of m/6 NaCl solution, the intravenous injection of very small quantities of pilocarpine caused a very marked diminution in the quantity of urine excreted. The subsequent injection of atropin into the blood counteracted the effect

[1]Journal of Physiology, Vol. XV, p. 433, 1894. Arch. f. Physiologie, du Bois-Reymond, S. 117, 1894.

[2]Arch. f. exp. Pathol. u. Pharm. Bd. 36, S. 411, 1895.

[3]Arch. di farm. e terap. 7, 1899.

[4]Grundniss der Arzneimittel-lehre. Leipzig, 1883, p. 66.

[5]Text-book of Pharmacology and Therapeutics, 1903.

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of the pilocarpine, so that the flow of urine returned to its previous rate. Atropin injected by itself was found to frequently have no effect when the quantity of urine resulting from the saline infusion was great. Sometimes it slightly diminished the quantity of urine excreted. In such cases the injection of pilocarpine counteracted this effect and restored the flow of urine to its former rate. The results of these experiments may be seen in the following descriptions:

Methods.

In all of these experiments rabbits were used. As an anaesthetic 4-5 c.c. 1% morphine hydrochlorate solution were in each case injected subcutaneously. As is well known, morphine somewhat depresses the secretory activity of the kidney in the rabbit, possibly partly on account of the fall of blood pressure which it occasions.[1] Almost invariably, therefore, no urine at all is secreted by a rabbit for a considerable time after the administration of an amount of morphine such as that mentioned. In order to overcome this anuria m/6 NaCl solution was injected steadily into one of the veins of the ear. This was done by means of an ordinary pressure bottle and a hypodermic needle tied to the end of a long rubber tube, which was coiled about in a water bath kept constantly at 40° C. In this way a uniform flow of m/6 NaCl solution into the blood vessels was established, with a velocity of 2 c.c. per minute. The urine was obtained by opening the abdominal cavity sufficiently to allow the bladder to protrude. A purse-string suture was then made in the fundus of the bladder and the organ opened within the suture. A cannula was then firmly fastened in the bladder, and connected with graduates by means of glass tubing. In this way it was very easy to obtain all the urine secreted during each unit of time.

Usually the urine begins to flow after 30-50 c.c. NaCl solution have been forced into the blood. This flow gradually increases until the amount of fluid secreted is only slightly less than that injected in the same time. When the rate of flow thus became constant, the substances to be tested were injected intravenously along with the NaCl solution.

[1]W. H. Thompson. Arch. f. Physiologie, du Bois-Reymond, 1894, S. 117.

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Experiments.

As shown in the following table, the flow of urine which results from the injection of a fairly large quantity of NaCl solution is within a few minutes after the injection of pilocarpine hydrochlorate greatly decreased. The subsequent injection of atropin sulphate causes this flow to return to its previous rate:

TABLE I.

Time.	m/6 NaCl Injected Intravenously.	Urine Collected.
10:15-10:30	40 c.c.	1.0 c.c.
10:35	15	1.2
10:40	10	3.0
10:45	10	7.9
10:50	15	10.5
10:55	10	10.5
10:56	1/4 c.c. 1/20% pilocarpine intravenously.	
11:00	10	8.0
11:05	10	7.0
11:10	10	3.4
11:15	10	1.9
11:16	1/4 c.c. 1/20% atropin intravenously.	
11:20	10	2.4
11:25	10	5.0
11:30	10	4.6
11:35	10	4.6
11:36	1/4 c.c. 1/20% atropin intravenously.	
11:40	10	4.6
11:45	10	6.2
11:50	10	8.4
11:55	10	8.6

A similar result is seen in Table II, where, however, after the administration of atropin the flow of urine in a unit of time becomes greater than the quantity of fluid injected. This is due to the previous suppression of the flow of urine by pilocarpine and the consequent accumulation of fluid in the blood.

TABLE II.

Time.	m/6 NaCl Injected Intravenously.	Urine Collected.
10:20-10:35	20 c.c.	
10:45	20	1 c.c.
10:50	20	3.8
10:55	10	5.0
11:00	10	7.2
11:05	10	8.0
11:10	10	9.0
11:12	1/4 c.c. 1/20% pilocarpine intravenously.	
11:15	10	7.0
11:20	10	4.2
11:25	10	4.0
11:30	10	4.0

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Time.	m/6 NaCl.	Urine. Collected.
11:31	1/4 c.c. 1/20% atropin intravenously.	
11:35	10	8.5
11:40	10	9.0
11:45	10	13.0
11:50	10	12.0
11:55	10	12.0

When a larger quantity of pilocarpine is given the diminution of the flow of urine is more abrupt and more marked, as shown in Table III. Here no atropin is subsequently added, and it is shown in this table how the flow of urine gradually comes to approach its former velocity, the injection of fluid being kept approximately constant throughout, or slightly modified, according as little or much is being secreted.

TABLE III.

Time.	m/6 NaCl Injected Intravenously.	Urine. Collected.
10:40-10:50	40 c.c.	5.0 c.c.
10:55	20	6.0
11:00	20	10.0
11:05	20	18.0
11:10	20	15.0
11:15	15	13.0
11:17	1/2 c.c. 1% pilocarpine intravenously. abruptly. Salivation marked.	Secretion stopped
11:20	20	4.0
11:25	15	6.0
11:30	15	2.5
11:35	10	2.0
11:40	20	3.0
11:45	10	4.0
11:50	10	4.0
11:55	10	5.5
12:00	10	7.0
12:05	10	8.5
12:10	10	9.0

It seems from this experiment that the diminution of the flow of urine is not due to an injury of the cells, since the kidney resumes gradually its former active secretion when the effect of the pilocarpine wears off, or when the pilocarpine has itself been excreted. Attention must be called, however, to the fact that it is impossible to keep the pressure exerted by the infusion of NaCl solution into the blood constant, since the diminution of the secretion would allow a greater accumulation of fluid in the blood. This can be partially controlled by varying the quantity of fluid injected according to the amount secreted.

When atropin is injected into the blood before any pilocarpine has been given, there is found to be a very slight diminution

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of the secretion of urine. This seems to be more marked when the total quantity of urine is small, as shown in Table IV. Here the subsequent injection of pilocarpine counteracts the effect of the atropin and increases the flow of urine again.

TABLE IV.

Time.	m/6 NaCl Injected Intravenously.	Urine. Collected.
10:20-10:30	30 c.c.	0.0 c.c.
10:40	10	2.0
10:50	10	3.4
10:55	10	2.0
11:00	10	2.4
11:05	10	3.8
11:10	10	3.6
11:15	10	3.2
11:20	10	3.4
11:22	1/4 c.c. 1/10% atropin sulphate intravenously.	
11:25	10	2.2
11:30	10	2.4
11:35	10	1.9
11:36	1/4 c.c. 1/10% pilocarpine intravenously.	
11:40	10	2.6
11:45	10	3.6
11:50	10	3.6

In the experiment shown in Table V much larger doses than those used in Table IV have exerted only the slightest, if any, effect. The small dose of pilocarpine, however, following this produces not the effect seen in Table IV, but that seen in Tables I and II. There is a marked diminution in the flow of urine.

TABLE V.

Time.	m/6 NaCl Injected Intravenously.	Urine Collected.
10:30-10:40	40 c.c.	1.0 c.c.
10:45	10	1.0
10:50	10	4.0
10:55	10	5.2
11:00	10	7.6
11:05	10	7.2
11:10	10	8.8
11:15	10	8.2
11:20	10	8.2
11:21	1/4 c.c. 1/10% atropin sulphate intravenously.	
11:25	10	8.2
11:30	10	7.4
11:35	10	7.8
11:36	1/2 c.c. 1/10% atropin sulphate intravenously.	
11:40	10	7.8
11:45	10	6.8
11:50	10	7.4
11:52	1/2 c.c. 1/20% pilocarpine intravenously.	
11:55	10	4.8
12:00	10	3.8
12:05	10	3.6

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One is tempted to suggest that in the experiment represented in Table V, where the quantity of fluid excreted almost equals that injected, the elimination must take place largely through the thin walls of Bowman's capsule. The cells making up these walls resemble secretory cells in no way, and atropin, which diminishes the activity of such typical secretory cells as those of the salivary glands, could not be expected to influence the passage of fluid through the cells of Bowman's capsule. Hence in Table V there is no diminution of the flow of urine. Similarly, in Table IV, where the quantity of fluid excreted is much smaller, a greater percentage of this fluid must be secreted by the convoluted tubules, which have a structure not unlike that of typical gland cells such as atropin controls. Hence, in this experiment the flow of urine was markedly diminished by atropin. If this be true, atropin influences only the elimination of fluid by the convoluted tubules, which is probably a true secretion. The passage of fluid through the capsule of Bowman, which is a process probably essentially different from the secretion of fluid from the salivary gland, for example, is not influenced by atropin.

The possible explanations for the action of pilocarpine are of some interest. Pilocarpine exerts an action on the flow of urine which is apparently the reverse of that which it exerts on the secretion from other glands, such as the salivary gland and the pancreas. This may perhaps be explained by the general action of pilocarpine on smooth muscle. It is well known that pilocarpine causes active peristalsis in the intestine, and often sharp constrictions which entirely shut off the lumen of the bowel. This may be readily demonstrated in a rabbit, and has been already described. It is conceivable that a similar phenomenon might take place in the pelvis of the kidney and in the other urinary passages. These passages have a muscular structure which resembles in a general way that of the intestine, and it is quite possible that the administration of pilocarpine might cause a constriction of the lumen so that the flow of urine would be partially obstructed. A similar series of events was described[1] in a discussion of the action of barium chloride on the flow of

[1]J. B. MacCallum: Journal of Experimental Zoology, Vol. 1, No. 1, 1904.

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urine, where it seemed almost certain that the sudden cessation of the flow of urine following the administration of barium chloride must be due to a constriction of the urinary passages. If this be true also for pilocarpine, the action of atropin in relieving the suppression of urine would be comprehensible, since atropin quiets the peristaltic activity and relieves the constrictions caused in the intestine by pilocarpine.

A second possible explanation of the diminution of the flow of urine caused by pilocarpine would seem to be the direct action of the pilocarpine on the muscular walls of the blood vessels of the kidney. It is conceivable that the pilocarpine might constrict the blood vessels of the kidney in such a way that the flow of blood through the kidney would be temporarily diminished. There seems to be no way of demonstrating this.

In the third place, the lowering of blood-pressure which pilocarpine usually causes must be taken into consideration. This fall of blood pressure is obtained only with relatively large doses, and in a number of experiments made in this laboratory by Dr. T. C. Burnett a quantity of pilocarpine much in excess of that given in the experiments described above produced no appreciable fall of blood pressure in the carotid artery. Since, however, measurements of blood pressure in the carotid do not necessarily indicate the condition which exists in the kidney, and since no determination of the changes in the volume of the kidney have been made with the administration of pilocarpine, the possibility that the decreased flow of urine caused by pilocarpine may be due to a fall of blood pressure must be considered.

Conclusions.

1. The flow of urine in a rabbit which has been increased by the intravenous injection of considerable quantitles of m/6 NaCl solution may be markedly diminished by the injection of small quantities of pilocarpine hydrochlorate solution.

2. This is due probably, not to any direct influence on secretion, but to a constricting action of the pilocarpine on the muscular tissue of the urinary passages, particularly of the pelvis of the kidney. The possible action of the pilocarpine on the

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muscular tissue of the blood vessels, and also on the blood pressure, must also be taken into account.

3. Atropin causes a slight diminution of the flow of urine. When, however, it is injected after the flow of urine has been diminished by pilocarpine it tends to counteract the effect of the latter substance, and to increase the flow of urine.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 14, pp. 113-123 May 18, 1905.

ON AN IMPROVED METHOD OF ARTIFICIAL PARTHENOGENESIS.

(Third Communication.)

BY

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I.

According to the method described in my two previous notes,[1] the unfertilized eggs of the sea-urchin *(Strongylocentrotus purpuratus)* were first submitted for two hours to hypertonic sea-water (100 c.c. sea-water plus 15 c.c. 2½ NaCl solution),[2] and were then treated with acetic or formic acid in order to produce a membrane. Since in the process of fertilization by sperm the membrane is formed immediately after the spermatozoon enters, it seemed to me that in the case of a complete imitation of the fertilizing effects of the spermatozoon by chemical means, the membrane formation should also constitute the first in the series of events. I had, however, met with the difficulty that when the membrane formation is caused first, and the eggs are submitted for two hours to the hypertonic sea-water afterward, they will not as a rule develop into larvae. I have since found that this difficulty can be overcome, and that the last postulate of a complete imitation of the process of fertilization by chemical means can also be satisfied,—namely, that the order of events should be the same in both cases. It is only necessary to submit the eggs which already possess a membrane for a considerably shorter

[1]See pages 83 and 89 of this series.

[2]This mixture will be referred to briefly as hypertonic sea-water.

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period than two hours to the hypertonic sea-water—from between 25 to 50 minutes—according to the temperature and differences in the eggs of different females.

My method of procedure is as follows: The eggs are first treated with one of the acids which cause the membrane formation. For this purpose 3 c.c. of a N/10 solution of a fatty acid, *e.g.*, acetic, propionic, butyric or valerianic, etc., are added to 50 c.c. of sea-water. The eggs remain in this solution for from ½ to 1½ minutes. When transferred back to normal sea-water they form a membrane which cannot be distinguished from a membrane of fertilization. The eggs remain in the normal sea-water for five or ten minutes, and are then put into a mixture of 100 c.c. of sea-water plus 15 c.c. 2½ NaCl solution for from 20 to 50 minutes. As the exact time at which they should be taken out from this hypertonic sea-water cannot be stated in advance, and does not depend upon the temperature alone, it is necessary to transfer lots of the eggs back into normal sea-water at intervals of three or five minutes. If they are left only a few minutes too long in the hypertonic sea-water the development is liable to be abnormal.[1]

If we proceed in this way the first segmentation of the eggs occurs (at a temperature of 19° C.) in about an hour and ten minutes after they are transferred from the hypertonic into the normal sea-water. The segmentation is in a certain percentage of the eggs perfectly normal from the beginning, and remains so throughout. The formation of micromeres occurs in these eggs just as in the fertilized eggs, and the rate of segmentation and development is also the same as in the fertilized eggs of the same female, and at the same temperature. The blastula cavity forms in these eggs as early and in the same way as in the fertilized eggs, and the blastulae begin to swim and rise to the surface as early as those developing from fertilized eggs. When the larvae which rise to the surface are collected and kept in a watch glass it is difficult to distinguish them from the larvae produced from the fertilized eggs. They develop into gastrulae and plutei at the same rate as the larvae that are produced from eggs fertilized

[1]A communication of this new method was made to the Society of Experimental Biology and Medicine of New York at the meeting of April 19th.

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by sperm. It is an easy matter to produce and collect an unlimited number of normal plutei from the eggs treated with this new method.

II.

If the experiment is carried out according to these directions, the various lots of eggs taken out at short intervals from the hypertonic sea-water will show the following differences in their segmentation and development. If at the temperature of 19° C. the first lot of eggs is transferred from the hypertonic sea-water into normal sea-water after about twenty minutes, no egg, as a rule, will segment in this lot. Possibly, no egg will segment in the next lot (with twenty-five minutes' exposure to the hypertonic sea-water), but in the lot with thirty minutes' exposure a certain small percentage of eggs may segment, though a little later than in some of the subsequent lots. Although the cell division may not be absolutely symmetrical, the result is as a rule a perfect two-cell stage. It would be hard to discriminate between the two-cell stage of these eggs and the two-cell stage of the fertilized eggs. A few of the eggs may segment in a less normal way. The quotient of the normally over the abnormally segmented eggs is very great indeed, certainly larger than one. If a few eggs segment in the lot of eggs with thirty minutes' exposure, many more will segment in the next lot, with thirty-five minutes' exposure. Here also the number of normally segmenting eggs is larger than the number of abnormally segmenting eggs, but only a fraction of the total number of eggs segment. The next lot, with forty minutes' exposure, will suddenly show a decided change. The majority, if not all, of the eggs segment, but the proportion of normally segmenting eggs is smaller than in the previous lot; as a rule about half of the eggs go into a normal two-cell stage, and the segmentation begins in this lot as a rule a little earlier than in the two previous lots. In the subsequent lots the number of eggs which segment decreases gradually although slowly, but the proportion of eggs which go into a normal two-cell stage decreases rapidly. Of the eggs which were exposed for an hour or more to the hypertonic sea-water none, or at least but few, begin their development with a normal two-cell stage.

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I have gathered the impression that the character of the first segmentation forecasts the relative number of normal embryos which will rise to the surface of the sea-water, and develop into normal plutei. The proportion of normal larvae in a lot of eggs seems to run parallel with the proportion of eggs which from the first show a normal segmentation, and I am inclined to believe that these eggs give most, if not all, the normal larvae which are obtained by this method. When the temperature is lower, and occasionally at a temperature of 20° C. the first lot of eggs to show segmentation is one with a slightly longer exposure to hypertonic sea-water than thirty minutes. In this case everything remains as stated above, except the time of exposure. It may require as much as 45 or 50 minutes' exposure to the hypertonic sea-water to cause development. It is possible to ascertain before the actual segmentation begins which lots have been over-exposed to the hypertonic sea-water. In the eggs of *Strongylocentrotus* the phenomena of nuclear division are already recognizable in the living egg. The eggs which are over-exposed show before the segmentation begins an irregular spindle formation and often multiple nuclei. Lots of eggs in which this phenomenon is very common give few or no normal larvae which rise to the surface. The sensitiveness of the eggs of the same female to hypertonic sea-water differs also, and for this reason one may find that in the same lot of eggs the optimal time of exposure was already exceeded for a few eggs, while for others it was barely reached.

III.

What is the relative share of the two agencies employed in the process of physico-chemical fertilization? If we call forth the membrane formation alone with the aid of a fatty acid in the way described above, without submitting the eggs to the hypertonic sea-water, they show after about 1½ to 2 hours (at 19° C.), the same optical changes which in the fertilized egg precede cell division, and which I take to be an indication of the spindle formation. While in the fertilized egg these apparent nuclear changes are followed immediately by cell division, this latter process does not occur at all, or only after from three to six hours or more, in the eggs where the membrane formation has been

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called forth, but which have not been submitted to the hypertonic sea-water. In almost all of the eggs, however, after about three hours, a process of disintegration begins which consists in a gradual breaking off of small droplets from the egg protoplasm. This disintegration begins always at one side of the egg (where the micromere material lies?), and as a rule leads to the complete disintegration of the egg in less than 24 hours. The same eggs if exposed after the membrane formation for from about 30 to 50 minutes to the hypertonic sea-water will not disintegrate, but begin to segment in about an hour and ten minutes.

If we submit eggs without a membrane to the hypertonic sea-water for from about 30 to 50 minutes, the eggs remain practically unaltered, and to all appearances do not differ from normal unfertilized eggs that have remained in normal sea-water.

It therefore seems that the changes connected with the membrane formation set also the apparatus for nuclear division into activity; but for some reason the cell division does not occur at all, or only very late. The subsequent treatment of hypertonic sea-water, therefore, serves at least two purposes: first, to render possible or to accelerate cell division; and second, to prevent the destructive processes which invariably follow the process of membrane formation by acid, if the egg is not submitted to hypertonic sea-water. I was curious to know whether the above mentioned destructive processes which follow the membrane formation by an acid were only a function of the membrane formation, or whether they are a general acid effect. It seems that the former is the case. I have already stated in a previous publication that if the eggs are taken out a little too early from the acidulated sea-water so that not all the eggs form a membrane when put back into normal sea-water, only those eggs disintegrate which have a membrane, while the other eggs do not undergo such a change, and remain alive. If the eggs are treated with an acid which is unfit for membrane formation, *e.g.*, nitric acid, the above-mentioned process of disintegration does not occur.

If eggs in which the membrane formation has been called forth by an acid are not submitted long enough to the hypertonic sea-water, they undergo the same process of disintegration as if they had not been submitted at all to the hypertonic sea-water.

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IV.

The method of artificial parthenogenesis described in the two preceding notes differed from the new one in the order in which the membrane formation and the exposure to the hypertonic sea-water followed one another. When the membrane is formed first, and the eggs are treated with hypertonic sea-water afterward, the exposure to the latter must be reduced to about half an hour or forty minutes. When the order is reversed, and the eggs are exposed to the hypertonic sea-water first, while the membrane formation is called forth afterward, the time of exposure must be raised to about an hour and a half or two hours in order to call forth a normal development. If with this latter order the eggs are exposed for half an hour or forty minutes to hypertonic sea-water, and the membrane formation is then caused by an acid, the eggs do not develop, but behave as if only their membrane formation had been called forth, *i.e.,* they begin to disintegrate after a number of hours. It is therefore obvious that we are not dealing with a mere algebraic addition of the independent effects of two agencies.

A parallel series of facts can be obtained if we try to combine the fertilization of eggs by spermatozoa and the treatment with hypertonic sea-water. The spermatozoon causes the formation of a membrane. If the eggs are first put for two hours into hypertonic sea-water and are subsequently fertilized with sperm, many more eggs will develop into larvae than if the eggs are first fertilized with sperm and then exposed for two hours to hypertonic sea-water The membrane formation caused by a spermatozoon increases therefore the sensitiveness of the egg to a subsequent treatment with hypertonic sea-water in the same way as does the membrane formation called forth by an acid.

V.

Since these experiments proved the great significance of the process of membrane formaton, it was of interest to ascertain which substances are fit to call forth this process. It is obvious that a complete knowledge of these substances must also include the substance which is responsible for the membrane formation immediately after the spermatozoon has entered the egg.

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I find that the substances which call forth the membrane formation in the egg may be divided into two groups. The one group includes hydrocarbons and certain products of substitution, the second group consists of certain acids. The two groups differ, in that the membrane formation occurs while the egg is in contact with the hydrocarbons, but does not occur while it is in contact with the acids, but only after it is transferred back into normal sea-water.

O. and R. Hertwig discovered that if sea-water is saturated with chloroform—only traces of which are soluble in water—the unfertilized eggs of the sea-urchin form a membrane when put into this chloroform sea-water.[1] Herbst found that benzol, toluol, and creosote, act similarly.[2] It seemed to me that possibly hydrocarbons in general might act in this way, and as a test I used amylene. It indeed called forth the membrane formation. This method of calling forth a membrane formation by hydrocarbons has a serious drawback, inasmuch as the eggs show a tendency to undergo cytolysis, and are killed. By the speedy transportation of the eggs into normal sea-water some may be saved.

The second group does not include all the acids, but as I have already indicated in a previous note, only certain acids. With one exception (CO_2) they are all monobasic organic acids. Di- and tri-basic organic acids, as well as the mineral acids thus far tested, are unfit for this purpose. The lower representatives of the fatty acid groups—formic, acetic, propionic, butyric, valerianic, and capronic acids—give excellent results. If 3 c.c. of a N/10 solution or the equivalent amount is added to 50 c.c. of sea-water, all the eggs of *Strongylocentrotus* will form a membrane, when after an exposure of from one-half to one minute to this solution (at a temperature of 19° C.) they are transferred back to normal sea-water. The higher fatty acids are very insoluble, and this causes certain complications in the experiment,—namely, a tendency to cytolysis; but aside from this tendency, caprylic, nonylic, caprinic, and laurinic acid[1] seem to give positive results.

[1]O. and R. HERTWIG: Untersuchungen zur Morphologie und Physiologie der Zelle, Heft. 5, Jena, 1887.

[2]HERBST: Biologisches Centralblatt, Vol. XIII, p. 14, 1893, and Mittheilungen aus d. Zool. Station Neapel, Vol. 16, p. 445, 1904.

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The oxyacids did not act as well as the fatty acids. It was necessary to expose the eggs for a longer time to the acidulated water and to add more acid. β—oxybutyric acid gave the best results among those tried. Oxyisobutyric acid was not so effective. When 9 c.c. of a N/10 solution of this acid were added to 50 c.c. sea-water, the eggs had to remain five minutes in the solution in order to form a membrane when put back into normal sea-water. Lactic acid had about the same low degree of efficiency, and glycollic acid was still less active.

In the aromatic group, benzoic and mandelic acids were tried: benzoic acid was as effective as the fatty acids, and mandelic acid was only slightly less effective. While it is easy to call forth the membrane formation in every unfertilized egg with the fatty acids, or with any of the monobasic acids mentioned above, the di- or tri- basic acids proved practically ineffective. Oxalic. succinic, maleic, tartaric, and citric acids were tried. After a long exposure only a few eggs formed a rather abnormal membrane when transferred back into normal sea-water, but this membrane formation was accompanied, or followed by, cytolysis. Neither could the mineral acids be used for this purpose. I have made a large number of experiments with HNO_3. The best results were obtained when 4 c.c. N/10 HNO_3 were added to 50 c.c. sea-water. When the eggs were taken from this solution after from two to five minutes, a certain percentage of them underwent membrane formation accompanied by cytolysis, which, of course, killed the eggs. Weaker solutions gave no membrane formation in a reasonable time. HCl and H_2SO_4 gave also practically negative results. In order to obtain approximately quantitative results with CO_2 I used it in the form of carbonated water. When 60 c.c. of m/2 NaClC solution saturated with CO_2 were added to 50 c.c. of sea-water, the mixture acted like the fatty acids for the process of membrane formation. If it were thinkable that CO_2 might act in this case as a monobasic acid (HOCOOH?), we might say that the presence of one, and only one, carboxyl group in the acid causes the membrane formation after the eggs are taken out of the solution.

[1]I am indebted for a number of these acids to my colleague, Professor Taylor.

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The fact that mineral acids gave only negative results suggested the idea of trying how a mixture of a mineral and a fatty acid would act. If 2½ c.c. N/10 HNO_3 are added to 50 c.c. of sea-water, and the eggs are left in the mixture for from 2 to 4 minutes, about 10% of the eggs form a membrane accompanied by cytolysis after they are put back into normal sea-water. If instead 2 c.c. N/10 HNO_3 + ½ c.c. N/10 propionic acid are added, all the eggs whch have been exposed to this mixture for about two minutes form a membrane after being transferred to normal sea-water. The membrane is not as perfect as if 2½ c.c propionic acid (without any nitric acid) had been used, but there is no cytolysis and immediate death of the eggs. When the eggs are put for one-half or one minute into a mixture of 3 c.c. N/10 propionic acid + 50 c.c. sea-water, they all form a membrane after being transferred to normal sea-water. If instead the eggs are put into a mixture of 1 c.c. N/10 propionic acid + 2 c.c. N/10/ nitric acid + 50 c.c. sea-water, they must remain in the mixture at least one minute before they form a membrane when transferred back into normal sea-water. The membrane is not as perfect as when propionic acid alone is used, but there is no cytolysis.

Since the eggs do not form a membrane when left in the acidulated sea-water, the idea presented itself that the presence of the acid (or the hydrogen ions) counteracted the membrane formation. It was easy to test this idea. When eggs were put into 50 c.c. sea-water to which 1 c.c. benzol or amylene had been added, all the eggs formed a membrane instantly, and then underwent cytolysis. This also occurred when care was taken to remove the nondissolved part of these substances. When, however, 3 c.c. of a N/10 fatty or mineral acid were added, the eggs swelled, but they neither formed a membrane nor underwent cytolysis. When the eggs were transferred early enough into normal sea-water they formed a membrane just as if they had been exposed to the acid alone, although their volume was larger, and they had evidently suffered.

The addition of formaldehyde, acetone, glycocoll, leucine, as well as of a number of salts, caused no membrane formation in the experiments thus far carried on, as long as the concentration of the sea-water was not changed.

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The question as to how the monobasic acids cause membrane formation requires quantitative determinations, of which I have made as yet but few. The minimum exposure of the eggs required for membrane formation is at a temperature of 20° C.

For 4 c.c. N/10 acetic acid + 50 c.c. sea-water, about 30 seconds.
3 c.c. N/10 acetic acid + 50 c.c. sea-water, about 45 seconds.
2 c.c. N/10 acetic acid + 50 c.c. sea-water, about 2 or 3 minutes.
1½ c.c. N/10 acetic acid + 50 c.c. sea-water, about 6 or 7 minutes.

The fact that part of the acid is neutralized by the sea-water must be taken into consideration for the calculation of these results.

It can also be shown that if the eggs are over-exposed the membrane will no longer be formed when they are transferred back to the sea-water. The over-exposure begins at 20° C. in a mixture of

10 c.c. N/10 acetic acid + 50 c.c. sea-water, after 45 seconds.
4 c.c. N/10 acetic acid + 50 c.c. sea-water, after 1½ minutes.
3 c.c. N/10 acetic acid + 50 c.c. sea-water, after 3 minutes.

The eggs which had been over-exposed could no longer either be fertilized with sperm.

VI.

It seemed of interest to ascertain whether it made any difference for the parthenogenetic development which substance was used for the production of the membrane. The eggs in which the membrane formation was called forth by fatty acids gave good embryos when subsequently exposed for about 30 to 50 minutes to hypertonic sea-water, although it seemed that the embryos in the eggs that had been treated with butyric and valerianic acid were slightly better than in those that had been treated with propionic or acetic acid. I was especially anxious to see whether the eggs in which the membrane formation had been called forth by a substance of the first group gave normal embryos. When the eggs were taken immediately after the formation of the membrane from the sea-water containing benzol, not all the eggs that had formed a membrane underwent cytolysis. When these eggs were subsequently treated in the way described above with hypertonic sea-water, they segmented, and some of them developed into plutei. As long a the formation of a membrane is induced by a substance which does not injure the egg

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too much, the subsequent short exposure to hypertonic sea-water may lead to the formation of an embryo. In regard to their vitality, and possibly their structure, the embryos may possibly differ according to the substance which is used for the production of the membrane. This, however, must be determined by further experiments.

All the facts mentioned in this and the previous communications indicate that the process of membrane formation is an essential, and not a secondary phenomenon in this method of artificial parthenogenesis. If eggs are treated with a fatty acid, but not long enough to call forth the process of membrane formation, or with an acid that is unfit to call forth this process, *e.g.*, nitric acid, a subsequent exposure of the eggs for about 30 or 50 minutes to hypertonic sea-water, will not cause the eggs to segment or develop. It should, however, not be overlooked that the process of membrane formation is in these experiments followed by the formation of a spindle, and that thus far these two phenomena have not been separated.

Five years ago I ventured the suggestion that the process of membrane formation is a process of coagulation. It is, however, obvious that the membrane formation in these experiments cannot be attributed to an acid coagulation, as in this case the membrane formation should occur while the eggs are in the acid, and not after they are taken out. Moreover, the fact that only certain acids act in this way also excludes such an opinion. From a closer observation of the process of membrane formation I am inclined to believe that it is due to a process of secretion, *i.e.*, the squeezing out under pressure of a liquid from the interior of the egg.[1] I am no more able to state the nature of the forces which underlie secretion in this than in any other case.

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[1]LOEB: This series, Vol. II, p. 73. Pflüger's Archiv, Vol. 103, p. 257, 1904.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 15, pp. 125-146 September 6, 1905

ON THE INFLUENCE OF TEMPERATURE UPON CARDIAC CONTRACTION AND ITS RELATION TO INFLUENCE OF TEMPERATURE UPON CHEMICAL REACTION VELOCITY.

BY

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Introduction.

The fact that temperature influences the heart rythm has been a matter of common knowledge for a long time. The first extended and systematic experiments along this line were made by Cyon[1] in 1866, who established that with increase of temperature the rate of the frog's heart-beat increases at first slowly then rapidly to the maximum rate, then with still farther increase of temperature, sinks first gradually then rapidly until it comes to a stand-still; that the limits of automatic contraction lie between —4° to 0°, and 30° to 40° C, while the maximum rate occurs as a rule between 30° and 34° C. He furthermore studied the effect of temperature changes upon the amplitude of the heart-beat and the duration of its systole, and, finally, calculated the relation between the amount of work done by the heart and increase of temperature, fixing the maximum between 18° and 26° C.

Langendorff[2] found that the cold apex of a frog's heart required twenty times as long to complete a rythmic cycle as a

[1] Cyon. Gesammelte physiologische Arbeiten. 1888, p. 36.

[2] Langendorff, O. Arch. of Anat. u. Physiol. (Physiol. Abt.) 1884. Sup. Bd. S. 33.

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warm apex; that isolated apices of frog hearts began to beat spontaneously under influence of increasing temperature within a period of time similar to the usual latent period of the entire organ; that irritability to a given induction shock and to chemicals (sodium chloride crystals) increased with increase of temperature.

The experiments of H. N. Martin[1] and of Langendorff[2] on the hearts of warm-blooded animals have likewise determined within more or less definite limits the maximum, minimum and optimum temperatures at which the isolated hearts of the dog and cat maintain rythmic contractions.

In his book on Brain Physiology Loeb[3] mentioned the possibility that the heart beat might be caused by a fermentative process which is going on constantly in the heart. He suggested to me that this idea could be tested by exact quantitative determinations of the influence of temperature upon the rate of the heart-beat. We know through Arrhenius how the velocity of a chemical reaction varies with the temperature. If the heart-beat were, as supposed, a function of a reaction velocity then the law of Arrhenius should also hold for the influence of temperature upon the rate of the heart-beat.

Method.

The heart of the Pacific Terrapin (Clemmys marmorata. Baird and Girard) was used throughout the investigation. The entire organ was excised with as little injury to the sinus as possible and fastened by its ventrical walls into a specially devised moist-air chamber which could be submerged in a water bath. Apertures in the cover of the chamber admitted a thermometer and a thread connecting the ventricular wall with a recording lever. A heart arranged thus in the moist-air chamber will be referred to throughout this paper as a *preparation*.

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[1] Martin, H. N. Studies Biol. Lab. John Hopkins Univ., Vols. II and IV., 1882 and 1890.

[2] Langendorff, O. Pflügers Arch. Bd., 66. 1897, S. 355.

[3] Loeb, Comparative Physiology of the Brain, etc., p. 23.

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Two principal series of experiments were made; one in which the temperature of the preparation was kept constant as long as the heart would beat, or as long as the experiment was continued. In all there were 26 hearts used in this series. In the other series the temperature constant of each preparation was changed during the experiment. Five different hearts were used in this series.

Continuous records of the heart-beats and of the time in minutes were kept upon revolving drums. The records of single preparations varied from one to eighteen hours.

From these records the following data were tabulated:

1. The age of the preparation at intervals of 5, 10, 15, 20, or 100 minutes apart, or any irregular interval at which time any remarkable phenomenon occurred.

At each successive interval:

2. The exact temperature of the preparation.
3. The number of minutes the beats were counted.
4. The average rate of the beats per minute.
5. The average amplitude of the contractions.

To this in the second series was added:

6. The number of minutes required for the preparation to reach the new normal rate produced by a change of temperature from one constant to another.

The temperatures, under which the preparations were experimented with, varied from 0° to 40° C at intervals of 2.5° apart. It was not always easy to keep the temperature exactly constant, but the variations did not amount to more than one degree from the standard set for a constant. For the most part the temperature was within a tenth of the desired constant. However, in any event the exact temperature was frequently observed and recorded in its proper place on the time-trace.

Results.

A. *Series I.*—As was to be expected there were noticeable variations of rate and amplitude in preparations put in the same bath, *i.e.*, subjected to the same temperature, other factors being as nearly the same as the conditions of experimentation

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permitted. The extent of this individual variation therefore was one of the first points to be settled. By selecting terrapin of nearly the same size and which had been reared together under the same conditions of diet and weather it was soon found out that the variation of rate in individuals was remarkably small, especially as to rate. As the work progressed it became more and more evident that faulty manipulation in removing the heart from the body, resulting in injury to the walls of the sinus and auricles, was the cause of the more pronounced cases of individual variation. It ought to be said here also that faulty excision of the heart affected the length of the life of the preparation and the amplitude of the contractions more than it did rate of heart-beat. There is some evidence, too, that season of the year affected the manner in which the rate was influenced by temperature.

The following tables show in a typical way the amount of individual variation that normally occurs between two preparations kept at a constant temperature:

When two preparations were put in a water bath and kept at 10°C the following rates were obtained:

Age of preparat'n in min.		5	10	15	20	30	40	50	60	80	100	120	140	160
Ave. rate per min.	No. 4	9.5	7.0	6.7	7.0	7.0	6.5	6.5	6.2	6.2	6.5	6.4	6.5	6.5
	No. 5	9.5	9.0	8.7	8.2	7.0	7.9	7.9	7.4	6.8	7.1	6.6	6.0	5.9
	Ave.	9.5	8.0	7.7	7.6	7.0	7.2	7.2	6.8	6.5	6.7	6.5	6.3	6.2

Two preparations kept at 20°C showed the following results:

Age of preparat'n in min.		5	10	15	20	30	40	50	60	80	100	120	140	160
Ave. rate per min.	No. 15	21.5	21.	19.	19.	16.	15.5	13.5	13.	11.	10.	8.	8.	7.6
	No. 16	21.	24.	18.	16.5	14.	15.5	16.	15.	14.5	10.	10.0	9.0	9.0
	Ave.	21.2	22.5	18.5	17.7	15.0	15.5	14.7	14.0	12.7	10.0	9.0	8.5	8.3

At 30°C we have the following results:

Age of preparat'n in min.		5	10	15	20	30	40	50	60	80	100	120	140	160
Ave. rate per min.	No. 12	48	48	48	48	41	5*	16	15	†	†	14.5	14.5	†
	No. 13	49	48	44	40	7.2	14.2	6	†	22.5	24.	7.5	14.	15.6
	Ave.	48.4	48	46	44	24.1	9.6	11	†	†	†	10.7	14.2	†

* The beats here were very irregular and feeble. As will be seen directly, the life of a preparation at temperatures above 25°C is rapidly shortened.

† The contractions here were too feeble to be counted, or had stopped entirely.

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On account of this close similarity in the rate of different hearts subjected to the same temperature it was decided that it would not be necessary to try many preparations at any one temperature. At the present writing two preparations have been tried at each of the following temperatures: 2.5°, 10°, 15°, 20°, 25°, 30°, 32.5°, and 37.5°. Three have been tried at 35°, and one each at 0°, 5°, 7.5°, 17.5°, 22.5°, 27.5°, and 40°.

The accompanying chart shows concisely the results, as far as rate is concerned, of the experiments with the heart preparations of this series, *i.e.*, with the hearts kept at constant temperatures. The figures in a vertical column in the body of the chart, indicate the rate of contractions of a single preparation at successive stages of the experiment. The data of the experiment, the number and temperature of the preparation are shown at the top of each such column. The figures in the vertical column on the extreme left indicate the "age" of the preparations at successive intervals of time; a horizontal series of figures, therefore, in the body of the chart represents the rates of the various preparations at a particular age. In all the spaces filled in regularly the number represents the average rate per minute as counted for at least two or three minutes at that particular interval. In the vast majority of cases the contractions were characterized by all the firmness, directness, and regularity that a heart-preparation is capable of exhibiting.

It will be seen from the chart that the rates of the two preparations at 2.5°, and also the two at 15°, differed considerably from one another, exhibiting marked individual variation. A study of the general character of the tracings of these hearts will convince any one that in each case the variation is due to injury of the heart tissues of one of the preparations. The March hearts at 35° show considerable similarity in spite of apparent injuries. The May heart, however, at the same temperature shoots away above the March specimens. In passing, attention may be called to the fact that all the May preparations showed greater strength and vitality, enduring high temperatures longer, and abrupt changes of temperature better, than the February or March preparations.

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No attempt was made to determine the length of time the hearts would continue to beat at the various temperatures; but in general it appears that those which were kept at the temperature of the air in Berkeley, or below it, beat the longest, while those kept at higher temperatures came to a stand-still sooner, according to the elevation of the temperature. Heat-rigor came on even at 30°, when the hearts were left in the bath long enough, the contractions ceasing with the heart in systole.

A casual glance at the chart also shows that the observation of other investigators is partially corroborated in the matter of maximum rate. E. G. Martin[1] found that the maximum rate of the isolated terrapin heart when perfused with Ringer's solution occured at 32°; Cyon[2] found the maximum rate to occur in the frog heart at 34°. While the highest rate reached by a single preparation in the present investigation occured at 37.5°, yet when the averages are taken, the highest rate is found to be at 35°. (See table of average rates.)

This maximum rate, however, holds only for the first five or ten minutes of exposure. At the end of twenty minutes it changes over to 40°; at the end of thirty minutes it has migrated to 30°; at the end of forty minutes it is at 32.5°; at 120, it is again at 30°; and for all the later stages it moves over to, and remains at, 15°.

B. *Series II.*—The experiments of this series were performed in order to answer the question as to how long it takes for a heart preparation to reach the new normal rate which is produced by a change from one temperature to another.

The moist-air chamber containing the heart preparation was suspended from an adjustable clamp on an iron stand. It was thus an easy matter to transfer it from one bath to another with as little loss of time as possible. Both the glass walls of the chamber and the air in the chamber being poor conductors of heat, it was necessary, when a change of temperature was desired, to immerse the chamber in a bath which was considerably higher (if an increase of temperature were wanted)—considerably lower if a decrease of temperature were wanted, than

[1] Martin, E. G. Amer. Journ. of Physiology. 11:382.
[2] Cyon. *Loc. cit.* p. 12.

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A CH

Age of the Preparation in Minutes	5/6 No. 33 0.0°
5	—
10	1.0
15	0.8
20	0.6
30	0.8
40	0.6
50	0.7
60	0.5
80	0.5
100	0.5
120	0.1
140	—
160	—
180	0.1
200	0.33
220	0.4
240	0.0
260	0.0
280	*
300	
320	
340	
360	
400	
500	
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700	

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the exact point of temperature constant sought for. When the thermometer in the chamber approached the new constant the preparation was at once removed and placed in a bath which had already been prepared and whose temperature was exactly at the degree wanted. Practice made it possible to bring about even extreme changes of the temperature within the air-chamber, and have the recording lever adjusted to the drum, within the space of a minute. This method was not adopted however until the latter half of the experiments in this series were begun. The method was useful in that it seemed to indicate that *the amount of time consumed by the preparation in reaching the new normal rate was largely the time required in changing the temperature of the air in the air-chamber, and not the time required for the heart tissue itself to react to the new temperature when once it was actually acquired.*

Preparations number 25, 26, 27, 28 and 29 were employed in this series. The results are shown in the following tables:

Preparation No. 25. 4/17/'05. Changing from 5° to 10° and *vice versa.*

Age of Preparation in Minutes	Temperature	Exposure, in Minutes	New Rate Established	Time in Minutes Required to Establish New Rate
30	5.0°C	30	6.0	—
38	10.0°	11	11.0	8
60	5.0°	30	6.0	19
95	10.0°	18	10.0	12
103	5.7°		6.0	7
		73		
158	4.0°		3.0	—
182	10.0°	27	8.5	10
209	5.8°	32	3.5	10
233	10.0°	40	7.6	7
277	5.0°	68	2.3	8
330	5.6°	—	2.5	—
341	9.0°	35	5.0	2
366	10.0°	—	5.8	—
Average No. of minutes required to establish new rate,				9.2

Preparation No. 26. 4/17 and 18/05. (*a*) Changing from 12.5° to 7.5°, (*b*) Changing from 17.5° to 12.5°, (*c*) changing from 15° to 20°, and *vice versa.*

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	Age of Preparation in Minutes	Temperature of Preparation	Exposure, in Minutes	New Rate Established	Time in Minutes Required to Establish New Rate
(*a*)	20	12.5°C	20	9.4	—
	30	7.5°	29	3.8	9
	58	12.5°	47	5.0	8
	107	7.5°	14	2.3	10
	125	7.3°	18	1.8	—
	139	12.5°	36	3.6	10
	180	7.5°	40	1.8	11
	218	12.5°	16	3.3	9
(*b*)	923	17.5°	13	7.0	10
	937	12.5°	19	4.0	9
	955	17.5°	30	6.5	7
	985	12.5°	33	3.6	8
	1021	17.5°	41	6.2	7
	1064	12.5°	52	3.6	7
(*c*)	1114	15.0°	42	5.0	5
	1158	20.0°	25	9.0	7
	1180	15.4°	24	5.0	4
	1205	20.0°	30	8.0	5
	1235	15.0°	70	4.5	5
	1311	19.9°	50	7.6	6
Average time required to take on new rate,					7.6

Preparation No. 27. 4/19/05. (*a*) Changing from 5° to 15°. (*b*) from 7.5° to 17.5°, (*c*) from 15° to 25°, and *vice versa*.

	Age of Preparation in Minutes	Temperature of Preparation	Exposure, in Minutes	New Rate Established	Time in Minutes Required to Establish New Rate
(*a*)	20	15.0°C	20	18.	—
	50	5.0°	30	3.4	10
	73	14.9°	23	14.5	13
	90	5.0°	17	3.5	5
	115	15.5°	25	16.0	10
	140	5.0°	25	3.2	8
	160	15.0°	20	15.0	10
(*b*)	180	17.5°	20	18.0	1
	200	7.5°	20	4.5	8
	215	17.5°	15	17.5	7
	240	7.6°	25	4.1	8
	300	17.3°	60	17.0	5
	360	7.2°	60	4.5	5
	395	17.5°	35	16.5	10
(*c*)	430	15.0°	35	13.2	6
	450	25.0°	20	26.5	9
	500	15.0°	50	13.0	6
Average time required to take on new rate,					7.5

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Preparations Nos. 28 and 29. No. 28, 4/22/05, changing from 25° to 5°, and *vice versa;* No. 29, 4/24/05, changing from 2.5° to 32.5°, and *vice versa.*

	Age of Preparation in Minutes	Temperature of Preparation	Exposure, in Minutes	New Rate Established	Time in Minutes Required to Establish New Rate
No. 28	13	25.3°C	20	43.0	9
	38	5.5°	30	3.5	16
	60	25.2°	11	31.0	6
	73	5.5°	23	3.5	10
	95	25.0°	12	29.0	9
	107	5.0°	12	2.5	10
	127	25.0°	19	28.0	8
No. 29	29	4.6°	15	3.2	—
	34	32.0°	6	44.0	4
	49	4.5°	9.5	5.0	9.5
	55	2.5°	27	0.8	6
	83	32.0°	18	43.0	5
	165	2.5°	69	1.4	—
	177	32.5°	18	34.0	5
	251	5.0°	34	4.3	8
	280	20.5°	40	17.0	12
Average time required to take on new rate, for No. 28,					9.7
for No. 29,					7.0

From these results it is seen that:

(*a*) The period of time required for a heart preparation to reach a new normal rate, caused by a change of temperature, and by the method employed above, is on an average from 7 to 9 minutes.

(*b*) By the proper manipulation this period of time is about the same whether the change in temperature is one of only 5 degrees or is one of 30 degrees.

(*c*) This period remains about the same, so long as the heart continues to beat rythmically, regardless of the age of the preparation.

(*d*) As was to be expected, exposure to a lower temperature prolongs the vitality of the preparation, enabling it to respond to an increase of temperature nearly as well at an advanced age as it does when in a fresh condition. This fact is shown most strikingly in numbers 28 and 29. In number 28 at an age of 95 minutes the rate, when the temperature is

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raised to 25°, is 29. After a rest at 5° (and until it is 127 minutes old) it resumes a rate of 28 when the temperature is raised again to 25°. Number 29 at an age of 34 minutes and a temperature of 32° has a rate of 44; after an exposure of about 35 minutes to a low temperature, *ca* 3°, and then immersion in a bath of 25°, at an age of 83 minutes, it beats at a rate of 43 per minute; a repetition of the treatment shows the same preparation at the age of 177 minutes to be beating at the rate of 34 beats per minute; at 280 minutes, after another routine of temperature changes of the same magnitude, this preparation has the rate of 17 beats per minute.

Now the preparations of Series No. 1 that were kept at 25°, when 90 minutes old, are beating respectively 6 and 8 times per minute; at the end of the 120th minute they are beating 4 and 5.5 times per minute. The 32.5° preparations of Series No. 1 at the age of 30 minutes have the rate of 20 each as against 44 of the preparation of Series No. II; at an age of 80 minutes they are going respectively at the rates of 15 and 12 as against 43 of the preparation in Series No. II at the same age.

(*e*) It has been observed[1] that sudden exposure to cold acts as a stimulus to the isolated heart, causing it to beat faster, at least for a short time. Although the air-chamber containing the heart preparation was frequently taken from a warm temperature and immersed in a bath of extremely cold temperature, *i.e.*, a bath at 0° or slightly below 0°, no such phenomenon was observed at any time in this series of experiments.

Theoretical Considerations.

1. Besides accelerating the heart rythm, a rising temperature is known to accelerate other life processes. The experiments of Clausen[2] on germinating seeds, of O. Hertwig[3] on developing frog eggs, and Merrifield[4] on metamorphosis of insects (all of whom made quantitative determinations of the influence of temperature upon the particular phenomena they had under

[1] Cyon, 1866, and Aristow, 1879. See Langendorff, O. Ueber den Einfluss verschiedener Temperaturen auf die Hertztätigkeit. Ergebnisse der Physiologie. Biophysik, II²: 517. 1903.

[2] Clausen. Landwirtsch. Jahrb. 1890. 19 : 893.

[3] Hertwig, 0. Arch. f. mikrosk. Anat. u. Entwicklungsgesch. 1898. 51:319.

[4] Merrifield. Reprints from the Proc. of the Entomol. Soc. of London. 1889.

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investigation) are often quoted. In offering an explanation of this accelerating effect of temperature writers generally have attributed it in part at least, if not wholly, to the effect of the temperature upon chemical changes which are supposed to be going on within the living tissues.[1] Hertwig, for instance, says that while he is "far from referring all the observed phenomena in the development of frog eggs to a chemical basis, yet the acceleration of the development of eggs under higher temperatures rests upon the fact (in his conception) that the synthesis of complicated nucleins takes place more quickly in the chemical laboratory of the cell under higher temperatures. While under decreasing temperatures the chemical work is gradually inhibited and finally brought to a standstill."[2]

However general the belief is that the acceleration of living processes brought on by temperature effects is at bottom an acceleration of chemical reactions, nevertheless no satisfactory experimental proof has as yet been brought forward in support of the idea.

2. Physical-chemists of late years have given us definite ideas concerning the influence which temperature has upon purely chemical reaction velocities. Van't Hoff[3] pointed out that a simple relation exists here. Arrhenius[4] showed that in many cases the relation can be represented by the formula,

$$Q_{t_1} = Q_{t_0} \cdot e^{A.(T_1 - T_0) : T_0 T_1}$$

where Q_{t_1} and Q_{t_0} represent the velocities at two different temperatures, t_1 and t_0; A, a constant; and T_1 and T_0 respectively the absolute temperatures.

The effect of temperature upon a chemical reaction, thus, is shown to be different from the effect of temperature upon known physical phenomena. As Arrhenius[5] has pointed out, the velocity of a gas molecule, according to the kinetic theory of gases, changes $\frac{1}{6}\%$ of its value with an increase of one degree. Likewise in-

[1] Cohen. Physical Chemistry for Physicians and Biologists. Transl. by Fischer, 1903. p. 50, ff. Hoeber. Physikalische Chemie der Zelle u. Gewebe. 1902. S. 315 ff.

[2] Hertwig, O. *loc. cit.*, p. 376.

[3] Van't Hoff, J. H. Vorlesungen ueber Theoretische u. Physikalische Chemie. 1898. Bd. I, S. 224.

[4] Arrhenius, Svante. Zeitschrift f. Physik. Chemie. 1899. Bd. 4, S. 226.

[5] *Loc. cit.*, p. 232.

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ternal friction is affected only 2% with an increase of one degree. On the other hand the velocity of a chemical reaction increases as much as 10% or 15% for each degree of temperature. At 6° the velocity of a chemical reaction is about twice its value at 0°; at 12° it is four times as much as at 0°, at 30° it is nearly thirty times as much as it is at 0°, etc. "This circumstance," Arrhenius goes on to say, "shows that reaction velocity as influenced by temperature cannot be explained by any changes of the physical properties of the reacting bodies."

To illustrate more clearly just what the nature of these characteristics is, the following tables of measurements from Arrhenius' original paper are reproduced. From the formula above it will be seen that the relation may be expressed approximately by saying that, if any two velocities of a chemical reaction going on at temperatures ten degrees apart be compared, the higher one will be found to be generally from two to three times greater than the lower one. In other words

$$\frac{\text{the velocity at } T_{n+10}}{\text{the velocity at } T_n} = 2, \text{ or } 3.$$

The values of this relation have been calculated for the tables of measurements and appear in the last column of each table.

(1) Decomposition of monochloracetic acid in an aqueous solution. van't Hoff. Etudes de dynamique chimique, p. 114.

Temp.	Observation	$\frac{T_{n+10}}{T_n}$
80.	2.22	
		$\frac{T_{90}}{T_{80}} = 2.2$
90.	6.03	
		$\frac{T_{100}}{T_{90}} = 2.8$
100.	17.3	
		$\frac{T_{110}}{T_{100}} = 2.5$
110.	43.6	
		$\frac{T_{120}}{T_{110}} = 2.4$
120.	105.	
		$\frac{T_{130}}{T_{120}} = 2.2$
130.	237.	

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(2) Substitution in $NaCH_2C100$ with NaOH. van't Hoff's Etudes, p. 113.

Temp.	Observation	$\frac{T_{n+10}}{T_n}$
70.	8.22	
		$\frac{T_{80}}{T_{70}} = 2.28$
80.	19.8	
		$\frac{T_{90}}{T_{80}} = 2.5$
90.	49.9	
		$\frac{T_{100}}{T_{90}} = 2.5$
100.	128.	
		$\frac{T_{110}}{T_{100}} = 2.3$
110.	305.	
		$\frac{T_{120}}{T_{110}} = 2.8$
120.	857.	
		$\frac{T_{130}}{T_{120}} = 2.5$
130.	2170.	

(3) Saponification of ethyl-acetate and sodium hydroxide. Warder. Berl. Ber. 14:1365. 1881.

Temp.	Observation	$\frac{T_{n+10}}{T_n}$
11.0	2.56	
		$\frac{T_{20.9}}{T_{11.0}} = 1.6$
20.9	4.99	
23.6	6.01	
		$\frac{T_{30.4}}{T_{20.9}} = 1.7$
27.0	7.24	
		$\frac{T_{34}}{T_{23.6}} = 1.7$
30.4	8.88	
		$\frac{T_{37.7}}{T_{27.0}} = 1.8$
34.0	10.92	
37.7	13.41	

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(4) Reaction of potassium chlorate and ferrous sulphate in presence of sulphuric acid. Hood. Phil. Mag. (5) 20, 1885.

Temp.	Observation	$\frac{T_{n+10}}{T_n}$
10.	1.00	
12.	1.21	$\frac{T_{20}}{T_{10}} = 2.5$
14.	1.46	$\frac{T_{22}}{T_{12}} = 2.4$
18.	2.11	
20.	2.51	$\frac{T_{24}}{T_{14}} = 2.4$
22.	2.96	
24.	3.59	$\frac{T_{28}}{T_{18}} = 2.11$
28.	5.08	
30.	6.04	$\frac{T_{30}}{T_{20}} = 2.5$
32.	7.15	$\frac{T_{32}}{T_{22}} = 2.9$

(5) Decomposition of dibromsuccinic acid in the presence of water. van't Hoff. Etudes, p. 112.

Temp.	Observation	$\frac{T_{n+10}}{T_n}$
40.	37.5	
		$\frac{T_{50}}{T_{40}} = 2.8$
50.	108.	
		$\frac{T_{60}}{T_{50}} = 2.6$
60.	284.	
		$\frac{T_{70}}{T_{60}} = 2.5$
70.	734.	
		$\frac{T_{80}}{T_{70}} = 2.7$
80.	2000.	
		$\frac{T_{89.4}}{T_{80}} = 2.2$
89.4	4540.	
		$\frac{T_{101}}{T_{89.4}} = 3.$
101.	13800.	

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(6) Inversion of cane sugar.

(*a*) Urech. Berl. Ber. 16:765.

Temp.	Observation	$\frac{T_{n+10}}{T_n}$
1.	0.434	
		$\frac{T_{10}}{T_1} = 0.5$
10.	0.217	
		$\frac{T_{20}}{T_{10}} = 4.6$
20.	1.00	
		$\frac{T_{30}}{T_{20}} = 4.3$
30.	4.30	
		$\frac{T_{40}}{T_{30}} = 3.3$
40.	14.60	

(*b*) Spohr. Zeitsch. f. Physik. Chemie. 2:195.

Temp.	Observation	$\frac{T_{n+10}}{T_n}$
40.	73.4	
		$\frac{T_{50}}{T_{40}} = 3.6$
45.	139.	
50.	268.	
		$\frac{T_{55}}{T_{45}} = 3.5$
55.	491.	

It will be noticed that while for the most part this quotient is quite constant, yet in the fourth example, the reaction of potassium chlorate and ferrous sulphate in the presence of sulphuric acid, the quotient varies from 2.1 to 2.9, or as much as 38%, within a range of 8 degrees; and that, in the case of the decomposition of dibromsuccinic acid (the 5″ example), the quotient varies from 2.2 to 3, or 36%. The following table, compiled by van't Hoff, shows to what extent this quotient may vary for different chemical reactions:

Reacting Bodies	Quotient
$PH_3 = P + 3H$	1.2
$C_2H_3O_2.C_2H_5 + NaOH$	1.89
$CH_2OHC_2Cl + KOH$	2.87
HPO_3 aq.	3
$OCS + H_2O$	3.68
The ferment, Emulsin	7.14

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3. If the effect of temperature upon reaction velocity, now, is one of such peculiar characteristics it would seem that there ought to be some way by which the same characteristics might be detected in the reaction velocity of a vital phenomenon, as influenced by temperature, provided that chemical reaction plays a prominent part in the phenomenon. If it is the case that chemical reaction is essentially the cause of cardiac contraction, the increase in the number of contractions in a given time ought to correspond somewhat closely to the increase of a chemical reaction velocity, *i.e., the relation of the velocity of the heart-rate ought to be similar to the velocity of a chemical reaction when influenced by temperature.* Instead of an increase of ⅙% or of 2% or any other widely different per cent, *the increase of the heart-rate ought to be (within certain limits) about* 10 *or* 15% *for each degree of increase in temperature. The number of beats for an increase of* 10° *ought to be* 2 *or* 3 *times.*

Following is a table of averages compiled from the large chart showing the various rates of heart-beat at different temperatures. For brevity only the decimal and demidecimal columns are shown here. The averages, too, at these temperatures approach more nearly the correct rates, since, excepting the rates under 0°, 5°, and 40°, they are made up of the records of more than one preparation.

THE AVERAGE RATES OF CONTRACTIONS PER MINUTE OF EXCISED TERRAPIN HEARTS at Various Temperatures, and at Successive Intervals of Time.

Age of Preparation in Minutes	Temperatures of the Preparations in Degrees Celsius								
	0°	5°	10°	15°	20°	25°	30°	35°	40°
5	—*	5.5	9.5	10.0	21.2	29.0	48.0	58.8	48.0
10	1.0	5.5	8.0	10.0	22.5	27.0	46.0	51.0	44.0
15	0.8	5.5	7.7	10.0	18.5	26.0	44.0	16.0	38.0
20	0.6	6.0	7.6	9.8	17.7	20.0	27.6	11.0	32.0
30	0.8	6.0	7.0	9.0	15.0	19.0	27.6	9.1	17.0
40	0.6	—	7.2	9.5	15.5	14.2	5.5†	8.2	†
50	0.7	—	7.2	9.7	14.7	11.0	19.0	6.1	—
60	0.5	6.0	6.8	9.0	14.0	9.0	20.0	4.6	—

NOTE.—* Where the dashes occur no observations were made. † The beats at this point were very irregular and feeble.

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The quotients for the rates in the table above have been calculated. These quotients should be compared with those obtained from the measurements of purely chemical reaction velocities.

The quotients of the rate at t_{n+10} divided by the rate at t_n are shown in the following table:

Age of Preparation in Minutes	$\frac{Rt_{10}}{Rt_0}$	$\frac{Rt_{15}}{Rt_5}$	$\frac{Rt_{20}}{Rt_{10}}$	$\frac{Rt_{25}}{Rt_{15}}$	$\frac{Rt_{30}}{Rt_{20}}$	$\frac{Rt_{35}}{Rt_{25}}$	$\frac{Rt_{40}}{Rt_{30}}$
5	—	1.8	2.2	2.9	2.2	2.0	1.0
10	8.0	1.8	2.8	2.7	2.0	1.9	0.9
15	—	1.8	2.4	2.6	2.3	0.6	0.8
20	—	1.7	2.5	2.0	1.5	0.5	1.1
30	8.7	1.8	2.1	2.1	1.8	0.4	0.6
40	12.0	—	2.0	1.5	†	0.6	†
50	10.2	—	2.0	1.1	1.3	0.5	—
60	13.6	1.5	2.0	1.0	1.4	0.5	—

†, The beats stopped or were very feeble at this point.
—, No observations were made at this point.

The average rates of heart-beat taken from the preparations of Series No. II are as follows:

Temp.	2.5°	5°	7.5°	10°	12.5°	15°	17.5°	20°	25°	32.5°
Rate	1.1	3.8	4.0	9.2	10.7	16	17.3	—	33	44

If the averages of the rates for the periods of time between 5 and 30 minutes be taken for each temperature of the preparations in Series No. I, and if these averages be added to the averages of Series No. II and their sums be divided by two, we have the following:

Temp.	0°	2.5°	5°	7.5°	10°	12.5°	15°	17.5°	20°	22.5°
Rate	.8	2.3	3.8	5.	8.2	10.7	13.5	17.6	18.5	21.4

Temp.	25°	27.5°	30°	32.5°	35°	37.5°	40°
Rate	29	42	35.5	41.3	40	40.3	35.8

The quotients of these average rates are as follows:

for 0° and 10°,	10.2
for 2.5° and 12.5°,	4.6
for 5° and 15°,	3.5
for 7.5° and 17.5°,	3.5
for 10° and 20°,	2.2
for 12.5° and 22.5°,	2.0
for 15° and 25°,	2.1
for 17.5° and 27.5°,	2.4
for 20° and 30°,	1.9
for 22.5° and 32.5°,	1.9
for 25° and 35°,	1.4
for 27.5° and 37.5°	0.9
for 30° and 40°,	1.0

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The most remarkable thing about this table, as well as the one which consists only of the quotients of the rates belonging to Series No. I, is that *the quotients, which approach most nearly the numbers found to be true in the case of purely chemical reaction velocities, are in the neighborhood of the more ordinary temperatures. The quotients which vary most from the chemical velocity quotient are those at the extreme ends of the series.*

4. The formula written above may be expressed also thus,

$$\log. k = -\frac{A}{T} + \text{constant},$$

where k is the velocity constant, T the absolute temperature; and A, a constant. If two values of k are known, then, of course, both of the constants in the formula can be calculated.

The values of A in the chemical reactions tabulated in this paper are as follows:

Hood. $KClO_3+FeSO_4+H_2SO_4$,	7,695
Warder. $C_2H_3O_2$. C_2H_5+NaOH,	5,579
Urech. Inversion of Cane-sugar,	12,800
Spohr. Inversion of Cane-sugar,	12,820
Hecht und Conrad. Ethyl-alcohol and Methyl-iodide,	9,850
Schwab. Monochlor-acetic acid into Glycollic acid and HCl,	13,280
Schwab. Monochlor-acetic acid and NaOH,	13,060
Schwab. Dibrom-succinic acid and H_2O,	11,110

The values of A vary considerably with different reacting bodies. The value of A, however, for different values of k in the same reaction are pretty nearly the same, as is seen in the two cases of the inversion of cane sugar cited above.

Assuming that the number of contractions per minute might be a rough measure of the velocity of a definite chemical reaction going on in the heart tissues, some of the values of A have been calculated from various values of k taken from the last table of average rates:

When $k_1 = 10.7$ and $k_2 = 21.5$, $A = 5905$
When $k_1 = 13.5$ and $k_2 = 29.0$, $A = 6523$
When $k_1 = 18.5$ and $k_2 = 35.8$, $A = 5922$
When $k_1 = 21.4$ and $k_2 = 41.3$, $A = 6021$

Two of these values vary as much as 8% from one another; yet considering the crudeness of our methods of measurement they are remarkably close to one another.

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Summary and Conclusion.

1. *When the rate of heart-beat of the Pacific Terrapin as influenced by temperature is compared with the velocity of a chemical reaction as influenced by temperature, a remarkable similarity is found to exist. The acceleration of heart-beat, due to temperature influence, progresses nearly in the same proportion as acceleration of the velocity of a chemical reaction when influenced by temperature.*

2. *Since the acceleration of a chemical reaction velocity goes on in a mathematical proportion not known to obtain in the case of any other physical phenomenon, and since this proportion is found also to express approximately the relation which exists between temperature and velocity of heart rythm for terrapin, we may conclude (in conjunction with much other familiar evidence bearing on the case) that acceleration in the case of the heart-beat is essentially due to acceleration of chemical reactions which go on within the heart tissues themselves.*

In addition to the above the investigation has shown also the following:

3. Hearts of the Pacific Terrapin when isolated and suspended in moist air at the same temperature beat, generally, at the same rate. Out of nine experiments there were three exceptions to this rule; in two of which one or both of the hearts showed evidence of having been injured in preparation, making it probable that the dissimilarity of rate in these two cases was not due to normal individual variation, but to defects of manipulation. In the third exception there was no evidence of injury; the heart going at the slower rate, however, had an amplitude nearly twice as great as the one which was beating at the faster rate.

In one case while two hearts experimented with in early spring beat at nearly the same rate (both being at the same temperature), a third heart two months later beat at a higher rate at the same temperature. The dissimilarity here was undoubtedly due to differences between the bodily condition of early and late spring terrapin.

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4. Of the temperatures between 0° and 40°, the minimum rate of contraction occurs at 0°, the maximum rate between 35° and 37.5°.

5. Of the temperatures between 0° and 40°, the lower the temperature, the longer the life of a heart-preparation continues. The higher the temperature, the shorter is the life of a heart-preparation.

6. The length of time required by a heart-preparation to reach a new normal rate, occasioned by a change of temperature, is about the same regardless of the magnitude of the change, or the age of the preparation, so long as they are within certain limits.

7. A sudden change from a higher to a lower temperature was at no time observed, during the present investigation, to act as a stimulus, causing the heart to beat faster or its amplitude to become greater.

In conclusion the writer wishes to express his heartiest thanks to Professor Loeb, whose untiring interest and sympathy in this work have been of invaluable assistance.

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ADDENDA.

A fuller table of averages from the chart showing the rates of heart-beat as influenced by temperature has been prepared and is herewith attached. Following it is added also a fuller table of quotients of $\frac{Rt_{n+10}}{Rt_n}$, which is based upon data contained in the tables of averages and the chart of the individual preparations.

TABLE I.

The Average Rates of Beat per Minute of the Excised Terrapin Heart at Various Temperatures, and at Successive Ages of the Preparation. (The rates in each column, excepting the 5.0° column, are the averages, of at least two individual hearts.)

Age of the Preparation in Minutes	Temperature of the Preparation in Degrees Celsius											
	0.0°	2.5°	5.0°	10.0°	15.0°	20.0°	25.0°	30.0°	32.5°	35.0°	37.5°	50.°
5	—*	—	5.5	9.5	10.0	21.2	29.0	48.0	49.5	58.8	55.5	48.0
10	1.0	—	5.5	8.0	10.0	22.5	27.0	46.0	48.0	51.0	55.0	44.0
15	0.8	3.7	5.5	7.7	10.0	18.5	26.0	44.0	36.0	16.0	34.0	38.0
20	0.6	3.5	5.5	7.6	9.8	17.7	20.0	27.6	29.0	11.0	31.0	32.0
30	0.8	3.1	4.9	7.0	9.0	15.0	19.0	27.6	20.0	9.1	26.0	17.0
40	0.6	2.9	3.0	7.2	9.5	15.5	14.2	5.5	17.0	8.2	†	†
50	0.7	2.7	4.2	7.2	9.7	14.7	11.0	19.0	20.0	6.1		
60	0.5	2.5	3.3	6.8	9.0	14.0	9.0	20.0	11.7	4.6		
80	0.5	2.2	4.1	6.5	9.3	12.7	7.0	11.5	13.5	5.3		
100	0.5	1.8	3.5	6.7	9.0	10.0	4.5	12.	15.3	†		
120	0.1	1.5	3.3	6.5	9.7	9.0	4.7	11.0	3.0			
140		1.3	3.7	6.3	10.6	8.5	6.1	14.2	†			
160		1.2	—	6.2	10.6	8.3	3.0	†				
180	0.1	1.0	—	6.2	10.6	9.0	4.6					
200	0.3	1.0	4.0	5.7	10.2	8.2	3.5					
220	0.4	1.0	2.6	5.6	10.2	6.2	3.0					
240	0.0	1.0	2.2	5.7	9.2	5.8	2.3					
260	0.0	0.9	2.0	5.8	9.6	5.8	—					
280	†	0.8	2.3	5.7	9.0	4.7	3.5					
300		0.8	2.2	5.1	9.0	4.1	3.0					
320		0.8	—	5.1	8.7	—	2.7					
340		0.8	2.1	4.6	8.3	—	2.7					
360		0.8	—	4.4	7.9	—	1.9					
380		0.7	—	4.6	8.1	—	1.1					
400		0.7	—	4.8	8.1	6.0	—					
500		0.5	—	4.4	7.2	5.6	—					
600		0.4	—	4.3	7.1	—	—					

REMARKS.—* No observations made in spaces containing a blank.
† The beats stopped, or became too feeble to count.

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TABLE II.

A Table of Quotients of the Formula, $\frac{Rt_{n+10}}{Rt_n}$. The numbers at the head of each column are the preparations (see the chart), from which the data for the calculations were obtained.

Age of the Preparation	Nos. 4, 5, & 33 $\frac{Rt_{10}}{Rt_0}$	Nos. 26 & 9 $\frac{Rt_{12.5}}{Rt_{2.5}}$	Nos. 6, 7, & 25 $\frac{Rt_{15}}{Rt_5}$	Nos. 27 & 34 $\frac{Rt_{17.5}}{Rt_{7.5}}$	Nos. 15, 16, & 4 $\frac{Rt_{20}}{Rt_{10}}$	Nos. 32 & 26 $\frac{Rt_{22.5}}{Rt_{12.5}}$	Nos. 10, 11, & 6, 7 $\frac{Rt_{25}}{Rt_{15}}$	Nos. 5 & 27 $\frac{Rt_{27.5}}{Rt_{17.5}}$	Nos. 12, 13 & 15, 16 $\frac{Rt_{30}}{Rt_{20}}$	Nos. 19, 20 & 32 $\frac{Rt_{32.5}}{Rt_{22.5}}$	Nos 31 17, 18 & 10, 11 $\frac{Rt_{35}}{Rt_{25}}$	Nos. 21, 22 & 35 $\frac{Rt_{37.5}}{Rt_{27.5}}$	Nos. 30 & 12, 13 $\frac{Rt_{40}}{Rt_{30}}$
5	—*	—	1.8	3.0	2.2	—	2.9	2.3	2.2	1.7	2.0	1.3	1.0
10	8.0	2.1	1.8	3.0	2.8	2.0	2.7	2.3	2.0	1.5	1.9	1.3	0.9
15	—	—	1.8	3.0	2.4	—	2.6	2.3	2.3	1.7	0.6	0.8	0.8
20	—	2.7	1.7	3.1	2.5	1.7	2.0	2.6	1.5	1.8	0.5	0.7	1.1
30	8.7	—	1.8	3.3	2.1	—	2.1	2.8	1.8	1.2	0.4	0.6	0.6
40	12.0	—	3.1	3.5	2.0	—	1.5	2.5	—	1.0	0.6	†	†
50	10.2	4.3	2.3	3.1	2.0	1.2	1.1	2.6	1.3	1.3	0.5		
60	13.6	2.1	2.7	3.8	2.0	2.6	1.0	2.3	1.4	0.7	0.5		
80	13.0	2.2	2.2	—	1.9	2.6	0.7	—	0.9	—	0.7		
100	13.4	2.7	2.5	—	1.5	2.6	0.5	—	1.2	—	†		
120	—	—	2.9	—	1.4	—	0.5	1.8	1.2	0.1			
140	—	2.8	2.2	3.7	1.3	3.2	0.5	1.5	1.6	†			
160	—	2.7	—	—	1.3	3.8	0.2	1.7	†				
180	10.0	—	—	—	1.4	—	0.4	1.6					
200	3.0		2.5	3.3	1.4	—	0.3	1.4					
220	2.5	3.2	3.9	—	1.1	—	0.3	—					
240	—		4.1	—	1.0	—	0.2	—					
260	—		4.8	4.3	1.0	—	—	—					
280			—	—	—	—	—	—					
300			4.0	—	0.8	—	0.3	—					

NOTE.—* No observations were made in the spaces containing a blank.
† The beats stopped, or became too feeble to count.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS
PHYSIOLOGY
Vol. 2, No. 16, pp. 147-158 September 12, 1905

ARTIFICIAL MEMBRANE-FORMATION AND CHEMICAL FERTILIZATION IN A STARFISH (ASTERINA).

BY
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1. It had always seemed to me that an understanding of the mechanism of fertilization could only be expected through the discovery of physico-chemical methods, which allow us to imitate the process of fertilization in all its essential details; as only in this case are we able to discriminate between the essential and secondary phenomena in this process. In three previous communications[1] I have stated the fact that the artificial production of a membrane in the egg of the sea-urchin seems to be an essential feature when a complete physico-chemical imitation of the process of fertilization in this egg is intended. The membrane formation can be produced in the egg of Strongylocentrotus purpuratus by two groups of substances, the one group (hydrocarbons like benzol or amylene) acting while the egg is in contact with the substance; the second group (consisting of certain acids, *e. g.,* fatty acids) calling forth the membrane formation only as an after effect, namely after the egg has been transferred back to the normal sea-water. Since the spermatozoon also calls forth a membrane formation in this egg it is possible, if not probable, that the spermatozoon produces its fertilizing effect entirely, or partly, by a substance belonging to one of the two groups mentioned before.

Two years ago I published a method whereby the egg of the sea-urchin can be fertilized by the sperm of all kinds of starfish.[2]

[1] Loeb: This series, Vol. II, pp. 83, 89, and 113, 1905.
[2] Loeb: This series, Vol. I, pp. 1, 39, and 85. Pflüger's Archiv, Vol. 99, pp. 323 and 637, 1903; and Vol. 104, p. 325, 1904.

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This method consisted in rendering the sea water slightly alkaline (or increasing its alkalinity slightly above the limit of alkalinity it naturally reaches). It is probable that this increase in alkalinity serves solely the purpose of making it possible for the starfish spermatozoon to fuse with the protoplasm of the sea-urchin's egg; and it is not very probable that the increased alkalinity is required for augmenting or modifying the fertilizing power of the starfish spermatozoon. This seems to follow from two facts, namely, that the treatment of the starfish sperm alone with alkaline sea water has little or no effect, and second, that if immediately after the entrance of the starfish spermatozoon into the sea-urchin's egg the latter is put back into normal sea-water the egg will develop. Since the entrance of the starfish spermatozoon into the egg of the sea-urchin also calls forth the formation of a membrane in this egg, it looks as if the fertilization of the starfish egg were caused by the same or similar chemicals which cause the fertilization of the egg of the sea-urchin. I have recently undertaken some experiments on the egg of the starfish for the purpose of deciding this question. The starfish used in these experiments was the Asterina from the Bay of Monterey. The egg of this starfish is especially well adapted for the investigation of this question, since it also forms a membrane upon the entrance of the spermatozoon.

2. The first task was to discover whether the substances which call forth the artificial membrane formation in the egg of the sea-urchin, act in the same way upon the unfertilized egg of Asterina. This is indeed the case; the only difference being that it requires, as a rule, a higher concentration of these substances in the case of Asterina, than in the case of Strongylocentrotus purpuratus.[1]

When the ripe eggs of Asterina are put into 50c.c. of sea-water which has been shaken with 1c.c. of benzol or amylene, all

[1] I may mention here that the concentration of fatty acids in sea-water required for the membrane formation is not the same in different species of sea-urchins. The addition of 3c.c. of N/10 acetic or butyric acid to 50c.c. of sea-water called forth the process of membrane formation in Strongylocentrotus pupuratus after a one-half minute's exposure, while for Strongylocentrotus Franciscanus this concentration of acid was too high. For the latter form 2 c.c. N/10 acetic or butyric acid had to be added to 50 c.c. of sea-water.

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the eggs will immediately form membranes while in the solution; and these membranes are identical in appearance with the membranes which are formed by the egg upon the entrance of a spermatozoon. If the eggs, however, are not taken out of the benzol or amylene sea-water immediately after the membrane formation, they will undergo cytolysis, i.e. the contents of the egg swell, are transformed into droplets, the pigment disappears, and the egg assumes the typical appearance of a "shadow." According to a current conception of this process in blood cells, the lipoid surface film of the egg has been dissolved or altered by the benzol, although the correctness of this explanation has not been proved.

If instead of a hydrocarbon we employ a fatty acid, no membrane is formed while the eggs of Asterina are in the acid sea-water; but the membrane is promptly formed after the egg has been transferred back into normal sea-water, provided the period of exposure to the acid has been neither too short nor too long. When 5c.c. N/10 acetic acid were added to 50c.c. of sea-water, no membrane was formed when the eggs remained in this solution less than one and one-half minutes (at 18° C.). But when left two minutes in this acidulated sea-water, they formed membranes after they had been transferred back into normal sea-water. Butyric acid and capronic acid give similar results. Hydrochloric and nitric acid have thus far given no satisfactory results. In some cases membranes were formed after the eggs were transferred back from the mineral acids into normal sea-water, but the membranes formed were not, as a rule, like those produced by a spermatozoon or by butyric or capronic acid, and were fewer in number.

The egg of the starfish is, as a rule, not yet mature when taken out of the ovary, but when put into the sea-water undergoes maturation in a rather short time, e.g. two hours or less. The immature egg has a large nucleus and the process of maturation is first indicated by the fact that this nucleus becomes less visible (by being partly dissolved in the protoplasm of the egg). Subsequently the polar bodies are thrown out and the egg then contains a nucleus which is considerably smaller than the original nucleus. If sperm is added to the eggs at the time when the

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original large nucleus is still visible, no membrane is formed, and such immature eggs are not fertilized, nor can they develop. Only when the nucleus has become invisible or reduced, can the sperm take effect. I have tried whether butyric acid or benzol could produce a membrane formation in the immature egg of Asterina; but this is not the case. Only after the large nucleus becomes invisible can the process of artificial membrane formation be called forth. The time when this is possible is practically the same for the spermatozoon and the butyric acid. I may mention that in my former experiments on the sea-urchin's egg I have also noticed that in immature eggs the natural and artificial membrane formation were impossible. It therefore seems as if some of the substances contained in the immature nucleus have to diffuse into the egg before the latter is capable of a membrane formation. This harmonizes with the fact discovered by Delage that enucleated fragments of a starfish egg cannot be fertilized by sperm unless the egg has undergone previously the process of maturation.

3. Our former publications on an improved method of artificial parthenogenesis in sea-urchins have shown that the membrane formation, or the process which results in the membrane formation, is an essential part of the normal process of fertilization, as well as of a complete physico-chemical imitation of this process. The question arises, is this also true in the case of the starfish? In order to decide this, we must compare the behavior of eggs of Asterina in which the membrane formation has been called forth by artificial means with those in which no membrane formation has been produced. Before we state this difference it may be well to recall the fact that the eggs of the starfish differ in an essential point from those of the sea-urchins, Strongylocentrotus and Arbacia. The eggs of the starfish show a slight tendency to develop spontaneously without any external influence. It perhaps expresses the difference partly (but only partly) when we state that the mature egg of Asterina is in a somewhat similar condition into which the eggs of Strongylocentrotus purpuratus are brought by a one and a half to two hours' treatment with hypertonic sea-water. I stated that sometimes

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none, sometimes a portion of one per cent, and sometimes more of the eggs of Strongylocentrotus treated by this method would develop. The development of these eggs was not as rapid and not so perfect as that of the fertilized eggs of the same form. But if the same eggs were afterwards treated with a fatty acid so as to call forth a membrane formation, a large percentage would develop, and in a number the development was as rapid and of the same type as that of the fertilized eggs. If the eggs of Asterina are allowed to mature in sea-water and are left to themselves, sometimes none, sometimes a fraction of a per cent, sometimes more, will segment and develop into larvae. But the development of these eggs is much slower than that of the fertilized eggs and, as a rule, the larvae are not so perfect and die sooner. If the eggs, however, are treated with butyric or capronic acid (or even with benzol) so as to cause a membrane formation without injuring the eggs, a development can be called forth which in kind and rate is practically identical with that produced by the entrance of a spermatozoon. As a rule, not all the eggs which form a membrane develop in such a way. A number of such eggs (which varies in different experiments) do not go into the two-cell stage at the proper time, but remain inactive for a number of hours (about five hours at a temperature of 18° C.). In one case the eggs of a female began to throw out their polar bodies between 10:30 to 10:40 A.M. At this time part of the eggs were fertilized with sperm, while part of the eggs were exposed for from one-half to a minute and a half to a mixture of 6c.c. N/10 butyric acid + 50c.c. sea-water. All the eggs in both groups (with the exception of a few immature eggs) formed a typical membrane of fertilization. In about two hours all the eggs that had been fertilized with sperm went into the two-cell stage, and at about the same time about 10% of those treated with butyric acid also began to segment. The latter continued to segment, only slightly more slowly than the eggs fertilized with sperm; but the segmentation-cells formed were equally perfect in both groups of eggs. Five hours after the treatment with butyric acid and the addition of sperm, respectively, the situation was as follows: The eggs that had been fertilized with sperm were

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all in the 16-cell stage. Of those treated with butyric acid, only 10% were segmented and they were in the 8- or 16-cell stage. The rest of the eggs were absolutely unsegmented and did not appear altered. But very soon afterward a change occurred in these latter eggs. Small, clear droplets were formed irregularly at various spots on the surface of their protoplasm, and the eggs broke up slowly into a mass of clear drops. Often not the whole egg but only a part underwent this process. For a time these eggs resembled eggs in about the 64-cell stage, with the difference that the single cell-like droplets were scattered more irregularly than in a normally developed egg. The fate of these two groups of eggs was, however, a different one. The normally segmented eggs that had been fertilized with butyric acid or with sperm developed the next day into swimming blastulae which were equally perfect in both cases. The butyric acid eggs, however, which had not segmented, but shown the droplet-formation (or myelin-formation) were black and dead the next day. In the eggs in which only part of the egg underwent this process of droplet formation, only *this part* was black the following morning.

The same observations were made in eggs of Asterina in which the membrane formation had been produced by benzol. One part of such eggs developed like normally fertilized eggs, while another part underwent the process of disintegration. The relative proportions of the normally developing eggs and those which underwent disintegration, differed very widely in various experiments, for reasons which I have not yet had a chance to determine.

The reader will have noticed the analogy between these observations on Asterina and the earlier ones on Strongylocentrotus. When the eggs of the sea-urchin were submitted to the process of membrane formation without any previous or further treatment with hypertonic sea-water, after about five or six hours a process of disintegration set in, which began also with the formation of small, clear droplets. When the eggs, however, had, previously or subsequently to the artificial membrane formation been submitted to hypertonic sea-water for the proper length of

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time, they developed like normally fertilized eggs. The end of the spawning season prevented me from trying the simple experiment of determining whether the eggs of Asterina which disintegrate six hours after the membrane formation will develop if submitted to a treatment with hypertonic sea-water for the proper length of time.

In the case of the sea-urchin it was found that if the eggs were treated with acid or benzol, but not long enough to produce a membrane formation in all the eggs, those eggs which had not formed a membrane did not, as a rule, undergo the normal type of development when previously, or subsequently, exposed to the hypertonic sea-water. It was of interest to try the corresponding experiment in Asterina. Eggs were put into a mixture of 5c.c. N/10 acetic acid + 50c.c. sea-water and left there one-half, a minute, and a minute and three-quarters. In the first two lots no eggs formed membranes, but in the third lot about 10% formed them. Nearly all of the latter eggs segmented normally and developed into the typical normal blastulae and gastrulae at about the same rate as the fertilized eggs. Those eggs which had formed membranes, but did not begin to segment regularly, showed at the proper time the peculiar disintegration mentioned above. Of the eggs which had not formed a membrane, a very small percentage segmented regularly, but much later than the eggs which had formed membranes. These eggs developed into smaller blastulae than the fertilized eggs or the eggs which had undergone the process of artificial membrane formation. The number of blastulae formed in the eggs without a membrane was smallest in the lot which had been exposed to the acid only half a minute. About ¼% of these eggs formed blastulae. Among the eggs of the second lot (one minute exposure to the acid) about 5% had formed blastulae. In the third lot, a large number of blastulae were found, and it was rather striking to observe the difference in the appearance of the blastulae formed in eggs with and without membranes. The former began to swim much earlier than the latter. Of those eggs which had neither formed a membrane nor segmented nearly all were dead, and had assumed a dark, almost black appearance in the place of the bright yellow

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color of the living eggs. Some of them had disintegrated under droplet- or myelin-formation, but the majority had not.

Among the control eggs which had been left in normal sea-water only a few had undergone segmentation (of a slow type) while the rest had perished and become black in less than 24 hours.

We have, therefore, two types of development in these eggs. One type is represented by the fertilized egg, and this type can be produced artificially in a number of eggs, at least, by calling forth the membrane formation by the above-mentioned artificial means. The second type is represented by the spontaneously developing egg in which no membrane formation has been called forth; these latter eggs begin to segment later, and possibly develop more slowly than the other eggs, and form larvae which are not as perfect as those belonging to the first type. The number of eggs which undergo the development of the second type can be increased by putting the eggs for some time into acidulated sea-water, even if this treatment does not lead to a membrane formation. This fact had already been established by the experiments of Neilson and myself, and of Delage. I am inclined to believe that for further research along these lines, especially where the raising of larvae is intended, the larvae of the first type are the more desirable ones; at least, as far as Asterina is concerned.

In the case of Strongylocentrotus I noticed that the higher fatty acids, such as butyric, valerianic, and capronic acid produced better larvae than acetic acid. The same seems to be the case with Asterina.

From all the facts mentioned here I have gathered the impression that the membrane formation, or possibly the process which results in a membrane formation, is an essential feature of the process of fertilization, not only in the sea-urchin, but also in, at least, certain starfish, *e.g.*, Asterina. It may seem pedantic to discriminate between the membrane formation and the process underlying it: but this discrimination is suggested by a suspicion on my part that the membrane formation is the result of a process of secretion of a liquid from the egg; and that this secretion,

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or the throwing out of certain substances from the egg, is the important feature, while the lifting up of the surface layer of the egg (the membrane formation proper) is only a mechanical consequence of this secretion, but of no importance in itself. This view is corroborated by the fact that under certain conditions the egg of the sea-urchin can be fertilized without being able to form a membrane. Several years ago I found that if the egg of the sea-urchin (Arbacia) is kept in sterile sea-water, free from bacteria, it can be fertilized after it has been kept in this way for about a week, and possibly longer. But when the egg has been kept under such conditions for more than 48 hours (at summer temperature), the egg no longer forms a membrane upon the entrance of the spermatozoon, although in every other respect its development is perfectly normal.[1]

4. The following facts may also be of interest for a discussion of the nature of the process of fertilization. In a former paper I have shown that the process of segmentation of the egg of Arbacia (and other animals) depends upon the presence of free oxygen, and that the substitution of hydrogen for air brings this process to a standstill very rapidly, if not almost immediately.[2] It is therefore obvious that oxidative processes are a prerequisite for the development of the egg. Three years ago[3] I called attention to the fact that maturation brings the starfish egg into a critical condition, in which one of two things happen: it is either caused to develop within a few hours after maturation, in which case it will live; or if this development does not occur, the egg will die very rapidly. I was able to show that this rapid death occurs only in the presence of oxygen. I have recently had a chance to verify these observations for Asterina. If we put the ovaries of an Asterina into a dish with water and allow the eggs to come out and remain in the sea-water without disturbing them mechanically, or otherwise, we find after 24 hours or less, the following three types of eggs: first, immature eggs (showing the large, unreduced nucleus) which are alive and

[1] Loeb: Pflüger's Archiv, Vol. 93, p. 59, 1902.

[2] Pflüger's Archiv, Vol. 62, p. 249, 1895.

[3] Pflüger's Archiv, Vol. 93, p. 59, 1902.

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live for a long time; second, mature eggs which have developed and are also alive; and third, mature eggs which have not developed, and which are dead and black. I have again verified the fact that this process of dying depends, like that of developing, upon the presence of oxygen. Where the atmospheric oxygen can diffuse freely to the mature eggs they die rapidly (unless they be caused to develop); but where the oxygen does not reach them they remain alive. When the eggs in a watch glass are dense and lie in several layers above each other, the mature eggs in the uppermost layer which do not develop are found disintegrated and black; while in the layer beneath, where the oxygen supply is cut off, in part or entirely (through the absorption of oxygen in the upper layer of eggs), the mature and undeveloped eggs are neither disintegrated nor discolored. It cannot be said that these eggs were in a dying condition at the beginning of the experiment and that they failed to develop on account of this condition; on the contrary, it can be shown positively that the reverse is true, namely, that these eggs die because they were not caused to develop at the critical period of maturity. This can be proved by the fact that if the eggs of the same female are divided into three lots, one of which is fertilized by sperm, the other chemically, while the third is not disturbed at all, all the eggs in the first lot and all, or a large portion, in the second lot, will develop and be alive the next day, and remain so; while those in the third lot will all be dead, with the exception of a variable but always small percentage which develop spontaneously. It is therefore obvious that oxidative processes occur in both kind of eggs, the mature unfertilized or resting egg of the starfish, as well as the fertilized or developing egg; but while in the latter egg the act of oxidation leads to development, in the unfertilized mature egg oxidation leads to death. From this we must draw the inference that the process of oxidation leads to the formation of different chemical products in the mature starfish egg with and without fertilization or development. The fertilized egg is a strict aërobe, but the mature unfertilized or non-developing egg of the starfish becomes an anaërobe, for which oxygen is a poison.

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It can indeed be shown that the chemical processes occurring in a mature egg which does not develop must be entirely different from those in the fertilized or developing egg. The essential effect of the entrance of a spermatozoon, as well as chemical fertilization upon the egg, is the starting of a series of cell divisions. It seems that from a physiological point of view this process consists preëminently in the transformation of protoplasmic into nuclear material. After the first nuclear division the mass of the chromosomes in each of the two nuclei is one-half that of the original nucleus. Then the chromosomes of the two nuclei grow until the mass of chromatine in each of the two nuclei is equal that of the original nucleus; and then another division of the nuclei, now into four, follows. This process is repeated until a definite ratio between the mass of chromatine and protoplasm is reached, and then the cell divisions stop. This has been proved by Boveri, who has shown that in a given species of sea-urchins the ultimate size of the blastomeres is always in proportion to the mass of chromatine present in the original nucleus.[1] It is obvious that the effect of a spermatozoon upon an egg consists in starting the transformation of protoplasm into nuclear material. The fact that this process comes to a standstill when the mass of the chromosomes and that of the protoplasm bear a definite relation to each other which, according to the observations of Sachs, Boveri, and others, is a constant in each species, suggests the possibility that the process involved in this transformation is a reversible one and that the process of segmentation stops when a condition of equilibrium is established between the chromosome material of the nucleus and the protoplasmic compounds from which it is formed. The fact that the growth of the mass of the nucleus and the diminuation of the mass of protoplasm is not continuous, but periodic, and interrupted by the phenomena of nuclear and cellular divisions, does not alter this conception. Experiments by O. Hertwig show that the influence of temperature upon the rate of development in the egg of the frog is similar to that found by van't Hoff and Arrhenius upon the velocity of chemical reactions.

[1] Boveri, Zellen-Studien, Heft 5, Jena, 1905.

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The processes of nuclear and cell division may well be secondary effects dependent upon the fact that the growth of each chromosome is limited, and that as soon as this limit is reached, other processes are started which lead to a division of the chromosomes. After this division is over the chromosomes are again able to grow.

We do not know how the spermatozoon starts or accelerates this process of transforming the protoplasm of the egg into the chromatine of the nucleus. It is possible that the spermatozoon carries something into the egg, *e.g.*, a positive catalyzer, which directly starts or accelerates this chemical process. If this is the case it is difficult to understand how the artificial membrane formation can act partly like a spermatozoon. A second possibility is that the spermatozoon destroys or eliminates a negative catalyzer or a condition from the egg whose presence inhibits or retards the transformation of protoplasmic into nuclear material. If this were the case, we might well understand why the secretion underlying the membrane formation might act so powerfully in starting the development. If the former view were correct,—namely, that the action of the spermatozoon consists solely in its carrying a positive catalyzer into the egg, it should also be possible for us to cause the unfertilized egg to develop by merely raising its temperature, since in this way the reaction velocity of the chemical processes is also accelerated. But while a higher temperature accelerates the development of the egg after it is fertilized I have thus far not been able to start the development in this way in the unfertilized egg, although I may be more successful in the future.

Whatever the decision in this case will be it remains true that oxidations lead to altogether different results in the developing and non-developing but mature starfish egg; and it is possible that in the developing egg the oxygen is consumed in the synthesis of chromatine from protoplasmic material, while in the mature but not developing egg these synthetical processes do not occur.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 17, pp. 159-162 October 7, 1905

ON THE INFLUENCE OF ELECTROLYTES UPON THE TOXICITY OF ALKALOIDS

(Preliminary Communication.)

BY

T. BRAILSFORD ROBERTSON.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

1. It has been suggested as a working hypothesis by Loeb and others that ions, when they diffuse into a living tissue, combine with some constituent of the tissue. For various reasons these compounds have received the name of *ion-proteids.*

2. Very numerous investigations by many observers have shown that the life-phenomena of organisms are very intimately dependent on the nature of the ions which are present in the tissues. It therefore appears probable that the properties of the ion-proteid molecule are very largely influenced by the ions which are combined in it.

3. In a previous paper[1] I have shown that there are many reasons for believing that the proportions of the different ion-proteids in a tissue depend on the relative *masses* of the ions which are acting upon it,—that the ions which are present in the greater mass take the larger share of the proteids.

4. In another paper, on the Influence of Electrolytes upon the Rhythm of the Heart, which has not yet been published, I have brought forward evidence in support of the view that the action between the proteid molecule and the ion which leads to the formation of an ion-proteid, consists in the simple addition of the ion to the proteid molecule.

5. Providing that this view is correct then, since ions move at different velocities, when ions are diffusing into protoplasm

[1] Trans. Royal Soc. of South Australia, 1905, vol. xxix, p. 31.

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that ion which has the highest velocity should form the major portion of the ion-proteid.

6. Hence, if the properties of the ion-proteid molecule depend chiefly upon the ion, when a saline solution is diffusing into a tissue, if the metal or positive ion has a higher velocity than the acid or negative ion, the resulting ion-proteid should be chiefly basic in character, since the greater part of the ion-proteid contains a basic ion. Similarly, if the acid ion has a higher velocity the resulting ion-proteid should be chiefly acid in character.

7. To test this hypothesis an investigation on the action of alkaloids upon protoplasm, which was subjected to the action of different saline solutions, was undertaken. It is very generally acknowledged that the actions of the alkaloids upon life-phenomena are primarily due to the formation of a compound between the alkaloid and some constituent of the protoplasm. Assuming that this constituent is the ion-proteid and that the alkaloid is only toxic *after* it has combined, then it seems probable that since all alkaloids possess more or less pronounced basic characters they should be more toxic when the ion-proteid has an acid character than when it has a basic character—since they would more readily combine with a molecule possessing acid properties than with one possessing basic properties. Some alkaloids, however, have both phenolic and basic characteristics owing to the presence of one or more hydroxyl groups as well as the unsaturated nitrogen atom. Such alkaloids should be toxic both when the ion-proteid is basic and when it is acid. Since the basic and phenolic characters are not usually present in the alkaloid to an equal degree, however, the toxicity should vary. In the special case in which the phenolic characters are pronounced, the alkaloid might be expected to be toxic chiefly when the ion-proteid is basic and much less toxic when the ion-proteid is acid.

8. A more extended series of experiments upon the action of Nicotine was undertaken, the organism used being a species of Tubifex. The experiments were as follows:—

I. Prepared finger bowls containing 55 c.cs of solution each as follows:—1. n/55$MgCl_2$; 2. n/55$CaCl_2$; 3. n/55CH_3COO-

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Na; 4. n/55 Na_2SO_4; 5. n/55 $MgCl_2$+n/2000 Nicotine; 6. n/55 $CaCl_2$+n/2000 Nicotine; 7. n/55 CH_3COONa+n/2000 Nicotine; 8. n/55 Na_2SO_4+n/2000 Nicotine.

In each a small number of the worms were placed. In twenty-five minutes nearly all the worms in 5 were motionless, in 6 the movements were very slight and some of the worms were motionless; in 7 the movements were slight, while in 8 the movements were sluggish but evident. In 1, 2, 3 and 4 the movements were normal and vigorous.

Full details of the state of affairs at different intervals of time will be published later,—but the order in which cessation of movement occurred in the solutions containing the alkaloid was as follows:—5, 6, 7, 8.

In the salts alone, on the contrary, cessation of movement took place first in 4,—while after five and one-half hours movements were more pronounced in 1 and 2 than in 3. Hence the greater toxicity of Nicotine in $MgCl_2$ cannot be referred to the greater toxicity of $MgCl_2$ alone.

II. Prepared finger bowls containing 55 c.cs each of solution as follows:—1. n/55 $MgSO_4$; 2. n/55 NH_4Cl; 3. n/55 KNO_3; 4. n/55 CH_3COOK; 5. n/55 $MgSO_4$+n/2000 Nicotine; 6. n/55 NH_4Cl+n/2000 Nicotine; 7. n/55 KNO_3+n/2000 Nicotine; 8. n/55 CH_3COOK+n/2000 Nicotine.

Cessation of movement took place first in 6 and 7; very soon after in 5 and much later in 8. In the salts alone death took place first in 4.

These experiments are being continued.

9. Thus it would appear, if our hypothesis is correct, that Nicotine combines chiefly, if not entirely, with acid ion-proteid, for cessation of movements takes place first in those solutions in which the acid ion of the salt has the higher velocity. This is in accordance with the structure of the Nicotine molecule, which does not contain an hydroxyl group.

10. These experiments upon the action of Nicotine are of special interest because of the localized action of Nicotine upon certain parts of the nervous system. Should our hypothesis turn out to be correct the action of Nicotine might be utilized to indi-

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cate the nature of the ion-proteids in different parts of the nervous system.

11. A series of experiments on the action of Quinine Hydrochloride upon Paramoecia was undertaken. The Quinine Hydrochloride was used in n/10000 solutions in n/50 solutions of the following salts:—$MgCl_2$, $MgSO_4$, $CaCl_2$, $BaCl_2$, NH_4Cl, KNO_3, CH_3COONa, Na_2SO_4 and CH_3COOK. It was found that the toxicity of quinine with different degrees of acidity or basicity of the ion-proteids had two maxima,—an absolute maximum when the ion-proteid was most basic and a relative maximum when the ion-proteid was most acid. The minimum of toxicity occurred when the ion-proteids were slightly acid. Quantitative experiments are being undertaken with a view to ascertaining the exact position of these maxima and minima and similar series of experiments are being carried out with other alkaloids.

12. This would appear to indicate that Quinine combines both with acid ion-proteid and with basic ion-proteid, but to a greater extent with basic ion-proteid. This is in accordance with the structure of the Quinine molecule which contains an hydroxyl group as well as the unsaturated nitrogen atom, while its phenolic characters are well pronounced.

13. These experiments upon the action of Quinine upon unicellular organisms are of especial interest because of the importance of a thorough knowledge of the conditions affecting the action of Quinine upon the plasmodium in malaria.

14. More extended quantitative experiments are being carried out, especially with reference to closely related alkaloids differing mainly in their hydroxyl or carboxyl group, and with reference to the influence of acidity or alkalinity of the saline medium in which the action of the alkaloid is tested.

15. I have only investigated the influence of salts upon the action of *alkaloids*,—but it is not inconceivable that the actions of other drugs with pronounced acid or basic characters may likewise be dependent upon the degree of acidity or basicity of the ion-proteids. Should this turn out to be the case this principle may be of considerable importance in therapeutics, since we would then be able to control and modify the actions of the drugs by the administration in various ways of appropriate saline solutions.

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 18, pp. 163-191 November 18, 1905

STUDIES ON THE TOXICITY OF SEA-WATER FOR FRESH-WATER ANIMALS (*GAMMARUS PULEX* DE GEER)

BY

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The following investigations were begun with the intention of obtaining some knowledge of the laws and special features of the *adaptation* of animals to the different concentrations of the surrounding fluid medium, at first of fresh-water animals to sea-water. On this occasion, however, it became necessary first to study thoroughly the *toxic phenomena of sudden changes of concentration* as well as their causes. The literature on this latter problem confines itself either to the determination of the length of life of fresh-water animals put into sea-water[1] (Paul Bert, Plateau, etc.), mostly made at only one concentration, or relates only to solutions of salts which are but partial constituents of sea-water (Yasuda, Massart, etc.). Investigations on the influence of the single salts contained in sea-water, especially as regards their relative concentration, which would prove especially interesting in their ecological relations, are wanting, as are also more exact quantitative determinations of the relation of the poisoning effect of sea-water to its concentration.

[1] The literature of this subject has been collected so often that a repetition would be superfluous. Among the easily accessible compilations may be mentioned: SEMPER: Existenzbedingungen der Tiere, Teil I, pp. 174 ff. and Anm. z. Kap. V, Leipzig 1880. DAVENPORT: Experimental Morphology, Part I, pp. 1–96, Part II, pp. 298–349, 1897–1899. VON FURTH: Vergleichende chemische Physiologie etc., pp. 622 ff., Jena 1903.

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Then, too, the proximate causes, or *the dynamics of these poisoning effects,* needed a closer analysis. Most of the earlier authors, and even von Fürth in his excellent book, are of the opinion that the poisonousness of sea-water for fresh-water animals consists mostly, if not entirely, in its *hypertonicity, i.e.,* that the *withdrawal of water* by the hypertonic solutions is responsible for the death of the animal. Although this idea of the more "physical" nature of those poisoning effects is rather probable, there are several considerations which can be advanced against this conception. It has been shown, for instance, by J. Loeb[1] that in the poisoning effects resulting from the opposite procedure, that of sudden immersion of marine amphipods (Allorchestis spec.) in fresh water or distilled water, the *removal of water* is not at all, as had been generally supposed, the only injurious factor, but that very probably some salts necessary for the maintenance of life are extracted. This view is especially supported by the investigations of Fredericq[2] and Quinton[3], the last of which was mainly responsible for demonstrating the permeability of the external membranes of marine animals not only to water but also to salts. Thus the idea of permeability to salts, which had already been known for plant membranes, artificial ones and those of higher animals, was extended also to marine organisms. The idea followed naturally that in the opposite case also, when fresh-water animals are subjected to sea-water, similar conditions might be found. The application of more exact quantitative methods showed indeed that the conception of the "pure physical"[4] nature of the salt effects does not correspond to the facts, but that, on the other hand, the toxic effects of sea-water are composed (in the same way as in Loeb's experiments the toxicity of fresh water) of several separate factors. By means of quantitative investigations it was at first attempted to obtain only a purely empirical analysis and interpretation of these effects.

[1] J. LOEB: Pflüger's Archiv, Bd. 97, p. 394, 1903.
[2] FREDERICQ: Bull. de l'Acad. Roy. de Belgique 1901, pp. 68 ff.
[3] QUINTON: Compt rend., T. 131, pp. 952 ff. 1900.
[4] VON FURTH: Chem. Physiologie, p. 629, Jena 1903.

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I.—On the Relation of the Toxicity of Sea-Water to its Concentration.

1. Methods. The material consisted of *Gammarus pulex* De Geer. Only copulating pairs were used in these experiments, in order, first, to obtain as homogeneous a material as possible; and, second, to determine if there is a difference in the behavior of the sexes. It may be supposed that these animals form a more homogeneous group as regards their physical make-up than others selected in any other manner, and that they represent normal and healthy organisms.

The concentration of the water was varied by adding in different experiments varying amounts of sea-water from San Francisco Bay, near Oakland, to the fresh water in which the animals had been confined for months. The salinity of this water was figured out from the exact value of the specific gravity determined with very accurate hydrometers by means of the formula

$$S = 1310 \left(Sp.\frac{17.5^\circ}{17.5^\circ} - 1\right)^{1}$$

The specific gravity was 1.018 at 17.5°, the salinity according to the formula 23.6 $^0/_{00}$. It appeared during the investigation that the range in the concentration of the water from fresh water to the undiluted sea-water was not sufficient to get exact results on the relation of toxicity to concentration; it accordingly became necessary to use still more concentrated solutions. I prepared these by evaporating Oakland sea-water in large flat dishes to half of its volume and then continued the experiments in the same way with this concentrated sea-water. The fresh water and sea-water were mixed as follows:

I. 90 parts fresh-water + 10 parts sea-water
80 parts fresh-water + 20 parts sea-water
70 parts fresh-water + 30 parts sea-water
etc. etc.

II. 90 parts sea-water + 10 parts concentrated sea-water
80 parts sea-water + 20 parts concentrated sea-water
70 parts sea-water + 30 parts concentrated sea-water
etc. etc.

[1] See *e.g.*, Supan: Physiche Erdkunde, 2 Aufl., p. 214, 1896.

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I preferred this method of evaporating the sea-water to the boiling of it, because it does not change so much of the bicarbonates of the sea-water into carbonates. Afterwards it appeared that the solutions prepared in this way also gave a decidedly alkaline reaction. A more thorough investigation of the common sea-water in the large aquarium jars in the laboratory showed, however, that this also reacted alkaline, especially in the daytime. Loeb and Cottrell[1] have found for normal Oakland sea-water that it is not alkaline, or but very slightly so. The former, however, showed that distinct alkalinity appeared if algae (ulva, etc.) were found in the jars.[2] The alkalinity of the sea-water I used was seen to be due, as in these other experiments, to the presence of algae.

After a number of preliminary trials, the following order of experiments was adopted as the best and most economical. The pairs were put with a pipette on a dry towel, which absorbed rather rapidly the adhering water and thus dried the animals rather completely. I found that a treatment of this kind does not influence the duration of life of the animals. On the contrary, I have seen animals still alive on the dry towel in the air about three hours after this procedure. As the investigations of Bullot[3] on the toxicity of distilled water have shown that the amount of fluid as well as the number of animals which were put at the same time into one dish have an influence on their duration of life, in the final experiments ten pairs were always put in 100 c.c. solution. I regret to say that in the last experiments I was obliged on account of lack of material to reduce this number to eight and six. I believe, however, that especially as the higher concentrations were used and the durations of life were correspondingly short, the influence of this change has not been a very important one.

As criteria of death, the stopping of the breathing movements and the power of spontaneous motion of the swimming legs and antennae after touching and pressing the animals rather

[1] Pflüger's Archiv, Bd. 99 p. 637, 1903.
[2] Pflüger's Archiv, Bd. 101 p. 347, 1904.
[3] BULLOT: University of California Publications, Physiology, Vol. I, 199 ff. 1904.

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strongly, were used. It could be observed that the movements of the gills persisted a longer time in lower concentrations than the movements of legs and antennae. This was the opposite of the behavior in more concentrated solutions, in which often five and ten minutes after the stopping of the movements of the gills spontaneous movements of the legs occurred.[1] The animals were put into the solutions simultaneously, then the duration of life of every single one was determined, and finally the average of all was figured out. In all experiments, no food was given, in order not to increase still more the variability of the results. Preliminary experiments showed that the animals were able to live without food about three weeks in pure fresh water (not distilled).

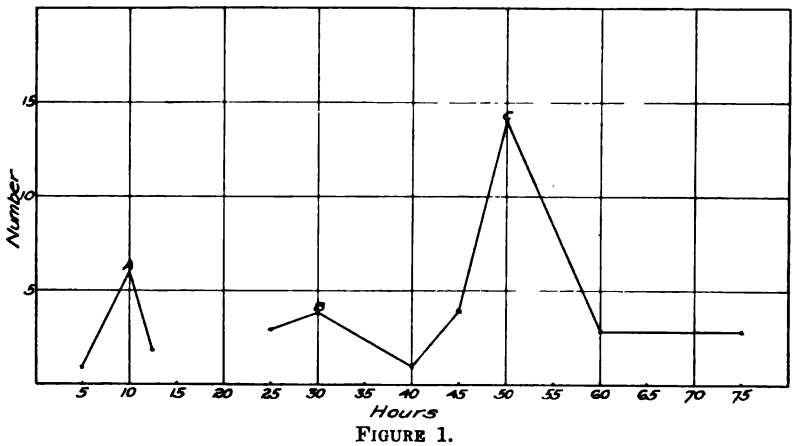

FIGURE 1.

Since the experiments were intended to be of a quantitative character, the factors of variability here involved had to be considered with especial carefulness. The first and probably most important factor consists in the variability of the animals themselves. In selecting only pairs, I tried to reduce this factor as much as possible. The variability which was nevertheless to be observed in the duration of life in the same solution is illustrated, for instance, by fig. 1, which shows the variability

[1] The movement of the gills can often be started again for a few seconds if, immediately after their stopping, the pressure of a pipette is applied to the anterior part of the body of the animal.

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of males in sea-water having a salinity of about 2%. The curve was obtained by plotting the figures obtained for the duration of life according to their frequency in the manner of variation statistics. Three maxima, *A, B* and *C*, are to be observed, of which A is relatively the most isolated, expressed in the figure by the breaking of the line. I believe that the significance of these three maxima is as follows: *A* probably represents the sick individuals, or those which have perhaps been hurt by the drying and transportation. *B* those which are just at the stage of shedding their skin; while *C* represents as the largest maximum the sum of the really normal and healthy animals. It should be added that only with the relatively diluted solutions, and correspondingly longer durations of life, such variability with several maxima was to be observed. At higher concentrations, only one maximum was normally found, apart from a very few animals that died very soon and which had apparently been injured.

A second factor in the variability is found in the uncertainty of the determination of the moment of death. I think I have limited this factor by the large number of observations and by the practice resulting from these.

In the third place, the influence of temperature is to be considered. Although I have not worked with a thermostat, I am convinced that the temperature has not varied more than 5° C during this first part of my investigations, on account, first. of the well-known equable climate of California, as well as, secondly, because the work was always done at the same time of day. Since, furthermore, the influence of temperature is to be especially investigated, it was here neglected. Other experiments made for other purposes at different times of the day (noon, afternoon, night, etc.) showed only a very insignificant variation of the durations of life within the limits of the temperature here to be considered.

In general, I believed that valuable quantitative results could be best obtained in these investigations by making as *many* experiments as possible and by repeating them often at different times and with different collections of individuals. Thus, for

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instance, for the determination of the curve which represents the relation of the toxicity of the sea-water to its concentration, I measured on different occasions over 900 durations of life.

2. Results. The results of the measurements of the relation of the toxicity of sea-water to its concentration are represented by the curves and tables here given. The abscissa represents the concentration, the ordinate the duration of life.

A striking fact was noticed even in the first experiments: the males and females behaved very differently as regards their tenacity of life. It was found that without exception the females are more sensitive to the influence of salts than the males. It may be mentioned here that this rule held also in all the experiments with mixtures of salts which are to be described later; the curve of the greater sensibility of the females ran often about parallel to that of the males, but sometimes it had also its specific features.

The curves on plate I begin with a concentration of 25.9 $^0/_{00}$, *i.e.*, a mixture of 90 parts Oakland sea-water and 10 parts concentrated Oakland sea-water. Fig. 2 shows why such a high concentration was selected for the starting point.

The experiments show that in the concentrations up to that of the sea-water the toxicity is still so small that a rather exact measurement, such as here desired, is not to be obtained. For it is to be expected, and it has been shown above, that the variability of the vitality, as well as of all single physiological properties, increases with the time during which the organisms are subjected to experiment or observation. The number of possible circumstances producing a variation in the duration of life increases inversely as the time. The concentrations up to 50 c.c. fresh water with 50 c.c. sea-water had no influence at all; the animals lived just as long as in pure fresh water. It was only from concentrations of 20 parts fresh water with 80 parts sea-water, and higher, that a visible toxic effect began to appear. But the figures thus obtained were still so large and varied so much that I excluded them from my experiments. A quantitative determination of the toxicity of those concentrations is possible only by working with an extremely large number of

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individuals (and then only with the help of graphic methods), or at higher temperatures.

As has been said, the toxicity for concentrations up to pure sea-water with a salinity of 23.6 °/₀₀ is zero or but slightly more. At the concentrations of from 23.6 °/₀₀ to 25.96 °/₀₀, however, it increases very rapidly for both sexes, but a little more rapidly for the females than for the males. The duration of life decreases in the case of the males from about 5–6 days to 46 hours with an increase of concentration from 23.6 °/₀₀ to 25.96 °/₀₀. In the case of the females, the duration of life decreases from about 29 to 5½ hours, *i.e.*, to about one-fifth or one-sixth of its initial value. For concentrations of from 25.6 °/₀₀ to 28.3 °/₀₀ the length of life of the females decreases only one hour and a half, while for the males the decrease is still 40½ hours, or one-eighth. At 28.3 °/₀₀ the decrease however takes place more slowly and gradually, until both curves from about 40.1 °/₀₀ on become straight lines or approach the abscissa assymptotically.

The difference between males and females decreases steadily with increasing concentration. While with normal sea-water the difference amounts to several days and at a concentration of 25.9 °/₀₀ to 41 hours, with the concentrated sea-water, (47.2 °/₀₀), it almost disappears, and at about 56.8 °/₀₀ it has entirely disappeared.

The exact figures are contained in the following table:

1. SEA-WATER.

Concentration in °/₀₀	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average duration of Life.
58.7	36	36	36.
47.2	46	48	47.
44.8	50	60	55.
42.5	50	75	60.5
40.1	53	79	66.
37.7	67	101	84.
35.4	77	120	98.5
33.0	86	147	116.5
30.6	127	192	159.5
28.3	185	326	255.5
25.9	328	ca. 46 hours	ca. 20¾ hours
23.6	ca. 29 hours	5 to 6 days	3 to 4 days

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I have not been able so far to figure out a formula that holds for the whole curve.

It is interesting that the experiments of J. Loeb on the toxicity of sea-water amphipods show an analogous result in so far as "up to a certain limit of dilution the duration of life decreases but little with additional dilution;—then, however, from a certain limit on, the decrease in the duration of life takes place very rapidly." It seems to me probable that the similar steep decline of the curves in both cases has a similar cause, consisting in both cases of a phenomenon of *coagulation*, which latter process occurs in some proteids, as is well known, at a certain increase of concentration of the electrolytes contained in a colloidal solution as well as at a certain dilution of it. Theoretical considerations of this kind, however, ought to be made later, and especially on the basis of more and different experiments.

II.—On the Dynamics of the Toxic Effects of Sea-water on Fresh-Water Gammarus.

It appeared to me that the most rational, although perhaps most tiresome, way to approach the dynamics of these toxic effects would be to carry on a systematic and detailed investigation of the toxic effects of the single chemical components of the sea-water, as well as of their possible variations and combinations. I believe the expectation was confirmed that especially larger series of similar measurements, which can be graphically represented, constitute the most profitable method of investigation for the present purpose.

The principal salts which come into consideration here are contained in sea-water according to vant Hoff's investigations in the following molecular relations:

1.

$NaCl = 100$	$KCl = 2.1$
$MgCl_2 = 7.8$	$CaCl_2 = 1.0$ app.[1]
$MgSO_4 = 3.8$	

[1] The amount of $CaCl_2$, which in contrast to the very constant amounts of the other salts varies in different sea-waters, is according to the investigations of Loeb about 1.0 molecular part for Oakland sea-water.

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At first the influence of these salts *alone* was studied in the relative concentration in which they occur in the sea-water.

The possible variations and combinations of these salts are the following:

2. *Binary Solutions.*

$NaCl + KCl$	$KCl + MgSO_4$
$NaCl + CaCl_2$	$KCl + MgCl_2$
$NaCl + MgSO_4$	$CaCl_2 + MgSO_4$
$NaCl + MgCl_2$	$CaCl_2 + MgCl_2$
$KCl + CaCl_2$	$MgSO_4 + MgCl_2$

3. *Ternary Solutions.*

$NaCl + KCl + CaCl_2$
$NaCl + KCl + MgSO_4$
$NaCl + KCl + MgCl_2$
$NaCl + CaCl_2 + MgSO_4$
$NaCl + CaCl_2 + MgCl_2$
$NaCl + MgSO_4 + MgCl_2$

4. *Quaternary Solutions.*

$NaCl + KCl + CaCl_2 + MgSO_4$
$NaCl + KCl + CaCl_2 + MgSO_4$
$NaCl + KCl + MgSO_4 + MgCl_2$
$NaCl + CaCl_2 + MgSO_4 + MgCl_2$

5. *vant Hoff's Solution.*

$NaCl + KCl + CaCl_2 + MgSO_4 + MgCl_2$

I.

Of those solutions which contained only one salt, NaCl was, of course, the most interesting. The curve, which represents the average values of males and females[1], shows the toxic effect of a pure solution of sodium chloride.

The curve has in general a similar form with that obtained by the use of sea-water. Here too there is a sudden, steep decline, which would appear still more distinctly in measuring the effects of still more diluted solutions. At the higher concentrations the curve also falls slowly and approaches the abscissa

[1] For the sake of clearness and the saving of space, the single values for males and females are given only in the tables.

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assymptotically. A remarkable difference, however, in comparison with the sea-water curve is that the durations of life are all of a considerably lower value. In other words, *a pure NaCl solution is much more toxic than the mixture of salts which represents the sea-water.* This strong toxicity of the pure NaCl solution is shown especially well by fig. 2, in which underneath the sea-water curves the curve for the toxicity of pure NaCl, in the same concentration in which it is contained in the corresponding concentrations of sea-water, has been represented. Although the NaCl solution is less concentrated than the corresponding sea-water, it has yet a considerably stronger toxicity.

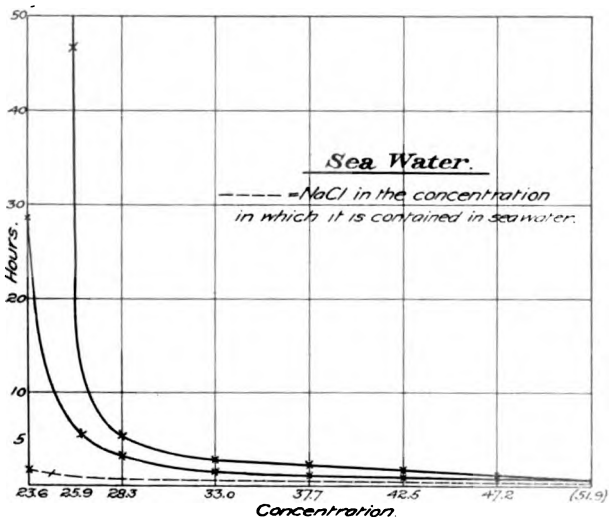

FIGURE 2.

To a certain extent also, this behavior corresponds to that of marine amphipods in diluted solutions. For J. Loeb[1] found that a NaCl solution isosmotic with sea-water proved of about the same toxicity as distilled water.

[1]J. LOEB: Pflüger's Archiv, Bd. 97, pp. 394 ff., 1903.

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The exact figures are contained in the following table:

TABLE 2. NaCl.

Concentration in ‰	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average duration of Life.
43.8	27	46	36.5
41.6	33	52	42.5
37.2	39	67	53.0
32.8	42	72	57.0
28.4	51	81	66.0
24.0	57	95	76.0
21.8	67	104	85.5
15.3[1]	—	—	243.0

It is evident from this toxicity of the pure NaCl solution, which is by far stronger than the toxicity of sea-water (fig. 2), that the addition of the other salts (KCl, $CaCl_2$, $MgCl_2$, and $MgSO_4$) must have a compensatory or antagonistic effect on the NaCl. The character of the further measurements will therefore consist in general of a construction of the effects of the different variations and combinations during which the special behavior of the single salts is not to be predicted.

So far as the behavior of the other single salts in distilled water is concerned, it was to be expected that on account of their small quantity their influence would not be very considerable. Hence only one measurement was made of each salt. The solutions contained a little more than the quantity which would correspond to the concentrated sea-water. The results are contained in the following table:

TABLE 3.

KCl.	100 c.c. dist. water + 4.4 c.c. 3/8 n KCl.	
	Females	216 minutes = about 3½ hours.
	Males	354 " = " 6 "
$MgCl_2$	100 c.c. dist. water + 15.6 c.c. 3/8 n $MgCl_2$.	
	Females	19–20 hours
	Males	46–48 "
$MgSO_4$	100 c.c. dist. water + 7.8 c.c. 3/8 n $MgSO_4$.	
	Females	26–28 hours
	Males	70–76 "
$CaCl_2$	100 c.c. dist. water + 2.0 c.c. 3/8 n $CaCl_2$.	
	Females	about 3 days
	Males	14–16 "

[1] The determinations of the toxicity of this diluted solution were made at the end of the experiments when the material had become scarce. Thus I could not determine the single values for males and females.

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The order of toxicity of the salts in their relative concentration is, therefore, KCl, $MgCl_2$, $MgSO_4$, and $CaCl_2$. KCl and $CaCl_2$ are the most different salts as regards their toxic effects. KCl is lethal in three to six hours, while $CaCl_2$, which is less than half as concentrated, shows its poisonous effects on the females only after about three days, and on the males only after over two weeks.

II. *Binary Solutions.*

Of the binary solutions only the combinations with NaCl were investigated. The toxicity of other combinations of the salts which occur only in small quantities in the sea-water appeared too small to make it probable that exact results capable of graphic representation could be obtained.

The curves on plate II show the results. It should be mentioned that to make the graphic representation clearer the curves of the average values of every solution have been displaced upwards for 60 minutes, so that to get the absolute values of the duration of life 0, 60, 120, etc., minutes are to be taken away from the corresponding ordinates.

The exact figures are:

TABLE 4. NaCl + $MgCl_2$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H[1]	25	46	31.5
E	30	49	39.5
C	40	73	56.5
B	52	89	71.5
A	—	—	235.0

TABLE 5. NaCl + $MgSO_4$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	33	53	43.0
E	37	63	50.0
C	49	98	73.5
B	68	108	86.0
A	—	—	173.0

[1] The letters indicate NaCl solutions of the following concentrations:
A=15.3‰ C=24.0‰ E=32.8‰ G=41.6‰ S=sea-water=20.7‰
B=21.8‰ D=28.4‰ F=37.2‰ H=43.8‰ DS=doubl. conc. sea-water=40.2‰

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TABLE 6. $NaCl + CaCl_2$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	28	37	32.5
E	34	59	46.5
C	43	71	57.0
B	82	108	85.0
A	—	—	339.0

TABLE 7. $NaCl + KCl$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	25	41	33.
E	36	49	43.
C	53	78	65.5
B	64	129	96.5
A	—	—	368.

The corresponding curves show in general the characteristics of the curves described hitherto: a steep decline with nearly straight and assymptotic course at the higher concentrations. They differ from the pure NaCl solution in the following respects: In the first place, the curves for the four binary solutions differ from each other. The curve for $NaCl + MgCl_2$ takes a particularly distinct position when compared with the curve for the pure NaCl solution. For the combination $NaCl + MgCl_2$ in the proportion in which these salts are contained in the sea-water is still more poisonous than NaCl alone. The difference holds, as the figures show, for the whole curve, *i.e.*, for all concentrations. Although this difference is not a very large one, its constancy for the whole curve shows that it is not due to errors in the observations or in the method of working.

The course of the curves of the combinations of NaCl with the other three salts is remarkably different. All three show higher absolute values; in other words, they indicate that they have a smaller toxicity than NaCl alone. This means *that those three salts (KCl, $CaCl_2$ and $MgSO_4$) have an antagonistic effect on the toxicity of NaCl.*

Arranged according to their compensatory properties, their order is: $MgSO_4$, $CaCl_2$ and KCl. Of these salts, KCl, with a duration of life of six hours and five minutes for the concentra-

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tion *A*, is the most favorable salt. This fact is rather astonishing, because it was found above that the pure solution of KCl, although contained in sea-water in the smallest quantity next to $CaCl_2$, has by far the most toxic effect of all the salts.

KCl and $CaCl_2$ in combination with NaCl behave very similarly, aside from the quantitative differences just mentioned, contrasting with $MgSO_4$, which has a rather isolated position. In the first place, the very high figures for the $MgSO_4$ curve at the higher concentrations are striking. For down to the concentration *C* they represent the highest values of all four curves, and approach very closely to the corresponding values of the sea-water curve. In addition there is a similarity with the $MgCl_2$ curve, insofar as in both the decline of the curve, when compared with the KCl and $CaCl_2$ curves, proceeds more slowly and gradually.

It is but another way of expressing the facts described if we define the difference in the behavior of the combinations NaCl + KCl, NaCl + $CaCl_2$, NaCl + $MgSO_4$, and on the other hand NaCl + $MgCl_2$ and pure NaCl, by saying that in the former group of solutions the decline of the curves begins later, and at a higher concentration than in the latter group.

It may be mentioned here that these experiments, together with the behavior to the pure NaCl solution, show that the toxic effects of sea-water on fresh-water animals are not at all of a purely "physical," *i.e.*, osmotic, nature. For if this were the case a pure NaCl solution could not be so much more toxic than a combination of it with the other four salts. Furthermore, the addition of the other salts ought to increase this toxicity in the ratio of their relative concentration—while quite contrary conditions have been found to exist.

III. *Ternary Solutions.*

The results of the experiments with ternary solutions are graphically represented in plate III. The exact figures are contained in the following tables:

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TABLE 8. $NaCl + MgCl_2 + MgSO_4$

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	26	47	36.5
E	38	59	48.5
B	50	98	74.0
A	—	—	ca. 5 hours

TABLE 9. $NaCl + CaCl_2 + MgSO_4$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	27	48	37.5
E	43	61	52.0
B	55	124	89.5
A	—	—	ca. 6 hours

TABLE 10. $NaCl + CaCl_2 + MgCl_2$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	22	41	31.5
E	41	60	51.5
B	53	79	66.0
A	—	—	ca. 5 hours

TABLE 11 $NaCl + KCl + MgSO_4$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	38	47	42.5
E	42	74	58.0
B	80	131	105.5
A	—	—	ca. 19 hours

TABLE 12. $NaCl + KCl + MgCl_2$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	38	46	42.
E	62	73	57.5
B	55 (?)	88	71.5 (?)
A	—	—	ca. 5 hours

TABLE 13. $NaCl + KCl + CaCl_2$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	39	57	48.
E	44	94	69.
B	72	107	89.5
A	—	—	ca. 13 hours

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The corresponding curves show again, in common with the curves described above, the rapid decrease at a certain concentration and a consequent straight and assymptotic course at higher concentrations. In contrast, however, to the binary curves, they all have a steeper and later decline, which indicates in other words, that all ternary solutions are less toxic than NaCl and the binary solutions.

In their details the effect of the single combinations is rather complicated. The best combination, *i.e.*, the one that is least toxic and in this respect most nearly approaches sea-water, is $NaCl + KCl + CaCl_2$. It is interesting that at the higher concentrations and correspondingly shorter durations of life the values of this curve not only reach those of the sea-water curve, but even surpass them (54 to 47 for the average of males and females). Furthermore, attention should be called to the extraordinarily rapid decline of this curve as well as to the relatively high concentrations at which this decline begins.

Next to this combination comes the mixture $NaCl + KCl + MgSO_4$. Here too is to be seen the tendecy of $MgSO_4$ to diminish the steepness of the curve which was to be observed in the case of the binary solutions. Compare especially the parts $\bar{B}E$ of the different curves. Here also the toxicity of a solution having a concentration corresponding to sea-water is about the same as that of sea-water alone (ca. 47 minutes).

The third place is taken by the combination $NaCl + CaCl_2 + MgSO_4$. In this curve also the specific influence of $MgSO_4$ in diminishing and retarding the decline is clearly to be seen, and especially so when the portion $\bar{B}E$ of this curve is compared with the same portion of the similar curve wherein $MgCl_2$ is substituted for $MgSO_4$. The other peculiarity of $MgSO_4$, namely, the high values for the high concentrations, has almost disappeared in these ternary solutions. Thus, although the H value is still one minute larger than the corresponding value of the combination $NaCl + KCl + MgCl_2$, it is five minutes less than in the solution $NaCl + KCl + CaCl_2$.

The other three curves are seen to be very similar, especially at the lower concentrations. All three are here still compara-

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tively toxic. Thus the duration of life at the concentration *A* is in remarkable agreement for all three combinations, being about five hours. It is interesting to see that this similar behavior is in all probability due to the circumstance that $MgCl_2$ has always been one of the constituent parts of the combination. As in the experiments with binary solutions, so also here, the strong effect of $MgCl_2$ in supporting the toxicity of NaCl is seen. The order of toxicity of the solutions which contain $MgCl_2$ is according to the other salts contained in the mixture:

$NaCl + KCl + MgCl_2$ as the best combination.
$NaCl + CaCl_2 + MgCl_2$
$NaCl + MgSO_4 + MgCl_2$.

In the last combination the specific influence of $MgSO_4$ is again to be observed in the slow bend of the curve as well as in the relatively long durations of life at higher concentrations.

Already at this stage of the investigation can be distinguished certain characteristics in the action of the individual salts; some of them supporting the action of NaCl and some of them antagonizing it. The discussion of these characteristics will, however, be postponed until after the description of the experiments.

IV. *Quaternary Solutions.*

Plate IV represents the experiments with quaternary solutions. The exact and detailed values are contained in the following tables:

TABLE 14. $NaCl + CaCl_2 + MgCl_2 + MgSO_4$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	29	37	33.
E	32	53	42.5
B	66	114	90.
A	—	—	ca. 5½ hours

TABLE 15. $NaCl + KCl + MgCl_2 + MgSO_4$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	29	41	35.
E	37	61	49.
B	68	92	80.
A	—	—	ca. 5½ hours

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TABLE 16. $NaCl + KCl + CaCl_2 + MgSO_4$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	30	42	36.
E	35	58	46.5
B	74	110	92.
A	—	—	ca. 34 hours

TABLE 17. $NaCl + KCl + CaCl_2 + MgCl_2$.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	28	42	35.
E	40	52	46.
B	47	89	68.
A	—	—	ca. 5½ hours

The curves representing these figures may be again divided into two groups: first, those which represent combinations with $MgCl_2$ and secondly, those without this salt (in this case, only one combination). This last curve, representing the combination $NaCl + KCl + CaCl_2 + MgSO_4$, occupies a unique position, in that it represents a much lower toxicity and a closer approach to the sea-water curve. The other three combinations, containing $MgCl_2$, are hardly less toxic than the corresponding ternary solutions (five to five and a half hours), but show again very characteristically the predominating role of the $MgCl_2$ effect by all three of them exhibiting in a rather astonishing way the same figure (5½ hours) for the concentration *A*.

The characteristic influence of $MgSO_4$ may also be recognized, except in the case of the combination $NaCl + KCl + CaCl_2 + MgSO_4$, where the favorable influence of the $KCl + CaCl_2$ is of such strength that the $MgSO_4$ effect cannot be detected. But this effect of the $MgSO_4$ may be seen very distinctly by the comparison of the other three curves, and especially by consideration of the steepness of the parts *AB* (90 to 5½, 80 to 5½, and in the solution without $MgSO_4$, 68 to 5½).

When these solutions are compared with the ternary solutions at the higher concentrations, the following two features are to be observed: first, a general decrease of the durations of life (from max. 48 to max. 36 minutes for the concentration *H*, from

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max. 69 to max. 49 for the concentration *E*, etc.; second, a similarity of the values of all combinations, or a disappearance of the specivity of the single salt effects. Thus, in the ternary solutions the most different values are 32 and 48 minutes, here only 33 and 36. The first circumstance is interesting in so far as by means of it this part of the curves is brought to a position which is a little *too low* when compared with the corresponding values for sea-water. It will be remembered that in the case of the ternary solutions the values for the higher concentrations were *too high* when compared with the corresponding values for the sea-water. But now the positive divergence from the sea-water is not so large as the negative one of the ternary solutions. This oscillatory fluctuation of the toxicity of the concentrated solution is very strange and unintelligible.

It is to be noted that in this case, as in those previously discussed, the decline of the optimal curve begins later and is also steeper than that of the other curves.

V. *van't Hoff's Solution.*

These experiments were made with a mixture of all five salts in the ratio in which they are contained in the sea-water, *i.e.*, with an artificial sea-water, which lacks only the traces of phosphates, iron, iodine, etc. The results are shown by plates V and VI and by the following table:

TABLE 18. van't Hoff's Solution.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
H	38	48	43.
E	59	80	69.5
B	78	119	98.5
A	—	—	ca. 96 hours (4 days)

The figures show that in general the van't Hoff's solution has about the same effect as sea-water of corresponding concentration. This is represented in Plate VI; the corresponding figures are given in Table 19.

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TABLE 19.

Concentration.	Van 't Hoff's Solution.	Sea-water.
H	43	43
DS	56	47
E	70	60
B	99	109
A[1]	ca. 4 days	less than 5 or 6 days
S	—	5 or 6 days

It should be mentioned that for the direct comparison of the same concentrations of both curves the figures given in Table 19 were not obtained from direct observation, but by interpolation from the observed values.

Very interesting and strange is the fact that, as these measurements show, the addition of $MgCl_2$ in van 't Hoff's solution brings about a decided improvement in all concentrations. This is in complete contradiction to its effect in all other solutions, single, binary, ternary and quaternary, in which it was contained. From these previous results it would have been expected that the optimal quaternary solution ($NaCl + KCl + CaCl_2 + MgSO_4$) would approach most nearly to the sea-water curve, and that the addition of $MgCl_2$ would increase the toxicity in this case also. The measurements, however, show, as mentioned, just the contrary.

With reference to the deviations of both curves, it should be said in the first place that they may have been produced by unavoidable experimental errors. This holds especially for the part $\overline{BH}$ of the curve for van 't Hoff's solution, for which, on account of lack of material, only one series of experiments (point E) could be made; and even this series contained but twelve individuals. It appears probable to me that a more exact measurement with more material would lower the value of the point E and annul in this way the difference of the curves at the higher concentrations, which is based only on this one figure.

This explanation, however, is probably not correct for the part of the curve which represents the lower concentrations: that is, at about the point B and especially to the left of it. Here I was able to make a larger number of more accurate

[1] The difference between A and S amount to but 0.2 ‰.

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measurements, so that the deviation is probably not due to faulty and unsatisfactory experiments. The deviation consists of a distinct lowering of the curve for van't Hoff's solution; that is, of an increase of its toxicity. This may be seen by a comparison of the curves as well as (and perhaps better) in Table 19. This difference was at first quite unintelligible to me, until I found what I think is its explanation in the following circumstance.

It has been mentioned above that the sea-water used in these experiments, and especially the concentrated sea-water, as well as all mixtures with it, had a decidedly alkaline reaction. On the other hand, my van't Hoff's solution reacted slightly acid to litmus, as it was made up of salts all of which had a small but measurable acidity. It is well known that acids belong to the strongest physiological poisons, and therefore I thought that the negative deviation of the van't Hoff's solution curve was due to this circumstance. I tried to modify the van't Hoff's solution by adding traces of alkali, especially of Na_2CO_3, or $NaHCO_3$ which very quickly becomes alkaline, which were used with success in similar cases by J. Loeb[1]. I succeeded completely in this attempt, investigating particularly the concentration *B*.

The results varied slightly according to the amount of the carbonate or bicarbonate added. The greatest improvement in a long series of experiments I obtained by an addition of 3 c.c. bicarbonate and of 0.5 c.c. carbonate to 100 c.c. van't Hoff's solution (corresponding to the concentration *B*). The results were in the first case:

females = 107, males = 124 minutes,

in the second:

females = 110, males = 163 minutes,

in both cases distinctly higher than those obtained with the acid van't Hoff's solution. The average values are 116 and 137, which are still a little high when compared with the corresponding value for sea-water which is about 110.

Although I was not able for the reasons mentioned above to carry out further experiments on other concentrations, I am convinced that a similar improvement would have resulted for

[1] J. LOEB: Pflüger's Archiv, Bd. 101, pp. 340 ff., 1904.

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these also. I think, however, that if my opinion that the rising of the van't Hoff's curve at the higher concentrations is one to experimental errors is right, the alkalinity or acidity of the solution can not have been of the same importance here as in the diluted solutions; especially when the strong inherent toxicity of those concentrations is considered. In an analogous way it might also be possible that the slow decrease and apparent disappearance of this difference in still more diluted solutions than *B* (see pl. VI) is due to the decreasing acidity of the van't Hoff's solution. According to this view the acidity of the van't Hoff's solution, increasing with the concentration of the solution, would cause an increase in toxicity, which would reach a maximum about point *B*, but which would be completely overshadowed at the high concentration by other toxic effects of the salts. Further experiments will have to show if this view is correct.

I do not think that the experiments here described are sufficiently numerous and varied to permit of a definite conclusion about the theory of toxic properties of the salt solutions investigated. As has been mentioned in the introduction, the first aim of these investigations was to determine if the idea of the purely "physical" or osmotical nature of the salt effects as maintained for instance especially by Paul Bert[1], is the one that corresponds best to the facts. The experiments described show distinctly that this theory is not at all right, at least not in its simple form which supposed the injurious effects to be due to the withdrawal of water. On the other hand the toxic effects are produced, more, in accordance with the view of the "older physiologists," by the summation of the specific effects of the single salts which do not work together osmotically but have their own very characteristic and rather constant features. There can, of course, be no doubt that besides those specific chemical or better physicochemical effects there are real osmotic phenomena. The above conclusions are directed only against the idea of the "simple" osmotic nature of these phenomena, an idea which is in my opinion not justifiable, because it is based on but relatively few and rather superficial experiments.

[1] See von Fuerth, Chemische Physiologie etc.

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Two facts should however be mentioned in connection with the osmotic theory of these toxic effects.

If we accept for the present the suitableness of the purely osmotic theory we must make at the outset the following restriction: *the toxicity of a hypertonic salt solution is not directly proportional to its hypertonicity.* Although I have not found the opposite of this sentence clearly stated in the literature, I have no doubt that most of the authors (including myself before the starting of these experiments) have been of this opposite opinion. If this idea were right, then the toxicity curve of the solution of a single salt ought to approximate, though perhaps it would not attain, the form of a straight line. But all the curves found in this investigation show in the contrary a decidedly rapid decline at a certain concentration, which, especially in the case of the sea-water, is of remarkable steepness and which corresponds to a critical point in the steady water withdrawal by the hypertonic solution. In other words, *there exists a critical concentration of the external solution at which the toxic effects of hypertonic salt solutions, hardly observable at lower concentrations, experience an extraordinary increase.*

It is furthermore important to note that even with this restriction the osmotic theory of these toxic effects does not correspond completely with the facts. The experiments show that this rapid decline of the curves by no means occurs at the same osmotic concentration of the external solutions, but that on the contrary under some circumstances (as shown by the comparison of NaCl and sea-water, fig. 2), *the more dilute solution is distinctly more toxic, i.e., reaches the critical point earlier than the concentrated one.* Even with this restriction then it is evident that we are dealing here with phenomena which are not at all of a purely osmotic nature.

I do not believe that it is possible to give a satisfactory explanation of the specific dynamics of these salts, or in other words of the characteristic forms of the single curves, on the basis of the experiments thus far made. Although I made a series of experiments with non-electrolytes, for instance cane-sugar, to study especially the part played by purely osmotic

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phenomena, I was not able to get a clear idea of the general conditions. The curve on pl. VI gives the average results; the exact figures are contained in table 20.

TABLE 20.

Concentration.	Duration of Life of Females in Minutes.	Duration of Life of Males in Minutes.	Average Duration of Life.
9/8 m.[1]	37	48	42.5
90 c.c. 9/8 m. + 10 c.c. 5/8 m.	38	53	45.9
80 c.c. 9/8 m. + 20 c.c. 5/8 m.	43	58	50.5
70 c.c. 9/8 m. + 30 c.c. 5/8 m.	48	62	55.0
50 c.c. 9/8 m. + 50 c.c. 5/8 m.	59	73	66.5
30 c.c. 9/8 m. + 70 c.c. 5/8 m.	69	87	78.0
10 c.c. 9/8 m. + 90 c.c. 5/8 m.	78	114	96.0
5/8 m.[2]	81	128	104.5

The table shows that

1. The cane-sugar curve is distinctly similar to the NaCl curve, but that

2. NaCl, especially in a concentration *isotonic* with cane-sugar, is distinctly *more toxic* than cane-sugar.

The latter fact may be seen from pl. VI. It should, however, be pointed out that both curves are not drawn in the isotonic position. To make such a comparison, it is necessary to displace the cane-sugar curve about one and one-half squares towards the right side, so that point S of the cane-sugar curve corresponds about to point C of the NaCl curve, etc. When this is done the lower toxicity of the cane-sugar is apparent. As examples of this difference between the toxicity of NaCl and cane-sugar, the following interpolated figures may be given:

Cane-sugar	105 min.	96 min.	78 min.	51 min.
NaCl	76 min.	about 72 min.	about 62 min.	about 42 min.

It is possible (and therefore I am not inclined to emphasize the difference in toxicity) that a 5/8 n. and a 9/8 n. solution of cane-sugar are not exactly isotonic respectively with sea-water of 23.6 ‰, 47.2 ‰, although these solutions have been used by J. Loeb in similar circumstances. Special experiments will have to decide this point.

[1] About isotonic with concentrated sea-water.
[2] About isotonic with Oakland sea-water.

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The most probable theory of these toxic effects appears to me to be that the deleterious effects of such hypertonic solutions consist in a coagulation of the plasma of the animals. This coagulation may be produced:

either by a certain increase in the concentration of the electrolytes dissolved in the interior of the cells, by means of the osmotic withdrawal of water,

or by the penetration of salts through the membranes,

or—which would probably agree best with the observed facts —by simultaneous effects of both kinds.

This idea is supported first by the more or less steep decline to be observed in *all* curves. Furthermore we are well acquainted with salts which have different effects on colloids according to different conditions of the solutions. This is especially true for the effects on albumin having different reactions, in which case we have antagonistic effects (Hoffmeister, Posternak, Pauli). It must be said, however, that the pure physical chemistry of these conditions has not been sufficiently worked out for their application to biological problems to lead to certain conclusions. Finally the idea that these salt effects are of the nature of coagulations is supported by the parallel experiments of J. Loeb mentioned above. In these also a similar critical point for the lowered concentration of the external solution and consequently a similar steep decline was observed —a behavior which would correspond to a coagulation on account of a lowering of the concentration of those electrolytes, the presence of which is necessary for the existence of the non-coagulated albumin solution.

Finally some experiments may be mentioned which show still another effect of solutions of different concentrations. I found during the experiments on the beneficial effects of small quantities of Na_2CO_3 that solutions of the same volume and the same alkalinity, which had been colored with a few drops of phenolphaleine, did not change their color simultaneously. If, for instance, 6/8 n. NaCl solution was made alkaline by a few c.c. of Na_2CO_3, and one part of this solution afterwards diluted

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to various concentrations, equal volumes being taken and the same number of animals (about twenty animals in one test-tube) put into them at the same time, even after a quarter of an hour a distinct difference in the colors of the two solutions could be seen. The color always disappeared first in the most concentrated solutions. After about half an hour, when in these experiments the animals are everywhere still alive, the most concentrated solution was nearly always completely decolorized. In other words *there is produced in the more concentrated solutions more of an acid,* the chemical nature of which I have not so far been able to determine, but which may be carbon dioxide, lactic acid, hippuric acid, etc.

These experiments are to be continued.

Summary.

The main results of this investigation are the following:

1. A curve for the toxicity of sea-water, of various concentrations up to 51.9%, was constructed as accurately as possible for the fresh-water gammarus. The experiments showed that the toxicity is different for both sexes, the females being in all cases less resistent than the males.

2. These toxic effects were investigated with reference to the particular effects of the single salts. The investigation was started with solutions containing only one salt and extended by testing most of the possible variations and combinations of the other salts contained in the sea-water. In this way the toxic effects of sea-water were analyzed, and were also built up by successive combinations of the single salts up to the van't Hoff's solution or artificial sea-water. The specific effects of the single salts are as follows:

NaCl in the concentration in which it is contained in sea-water is much *more toxic* than the relatively more concentrated sea-water itself.

KCl in combination with NaCl lowers the toxicity of the latter, and it does this more than any of the other salts. This is to be seen first in the increase of the durations of life as well as in the later beginning of the characteristic decline of the curves.

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$CaCl_2$ has a similar although not quite so strong antagonistic effect.

$MgSO_4$ counteracts also the toxicity of NaCl, although in a still lower degree than $CaCl_2$. In addition $MgSO_4$ has a characteristic and specific influence on the shape of the curves in so far as it makes the decline more gradual for *all* the solutions in which it is contained.

These three salts then have a distinctly antagonistic effect on the NaCl. $MgCl_2$ on the other hand increases the toxicity of the solution when combined with NaCl alone, as well as in every other combination (with exception of van't Hoff's solution).

The order of toxicity of the optimal solutions (taken from single, binary, ternary, quaternary, etc., solutions) is the following:

NaCl,
NaCl + KCl,
NaCl + KCl + $CaCl_2$,
NaCl + KCl + $CaCl_2$ + $MgSO_4$,
NaCl + KCl + $CaCl_2$ + $MgSO_4$ + $MgCl_2$,
or van't Hoff's solution.
Sea-water[1]

Several smaller deviations of van't Hoff's solution from sea-water are to be considered as produced in part by experimental errors, in part by the acidity of *van't Hoff's* solution which contrasts with the distinctly alkaline reaction of the sea-water used in these experiments.[2] The latter explanation was tested experimentally and found to be right.

3. For the bearing of these results on a possible theory of these toxic effects reference must be made to the text. The experiments showed, however, that in general the toxic effects of sea-water on fresh-water animals are not at all according to the current conception of a purely "physical" or osmotic nature, but that here as in the experiments of J. Loeb on the effects of diluted solutions on sea-water animals certain specific chemical

[1] (See Pl. VI).
[2] Also the lack of phosphates, carbonates, etc., has to be taken into consideration.

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or physico-chemical processes, which may be connected with osmotic phenomena, play the main role. These special phenomena probably consist in the coagulating effects of the salts.

4. Some preliminary experiments were described in which it could be observed that a production of acids of an unknown chemical nature by the animals increases rapidly with increasing concentration of the solution and perhaps takes part in this way in the toxic effects of the salts.

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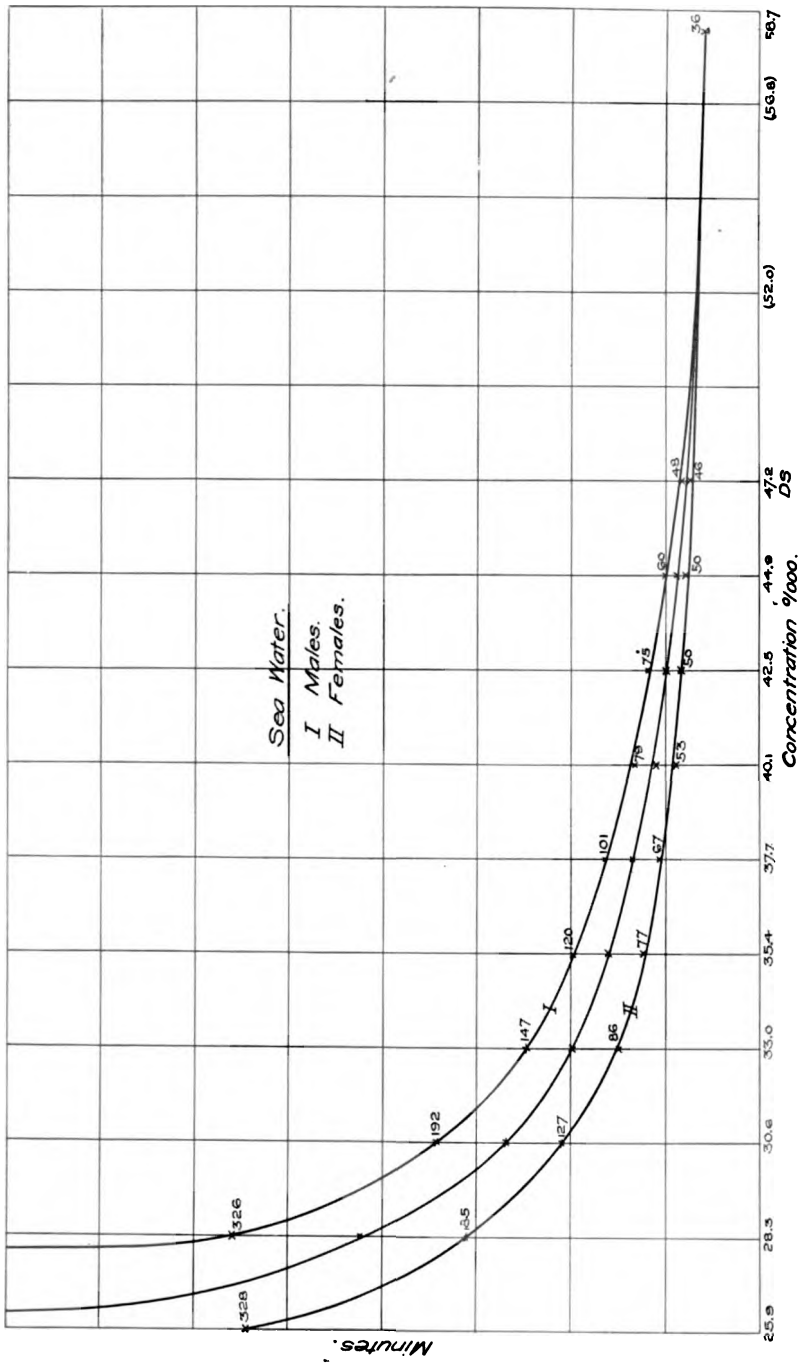

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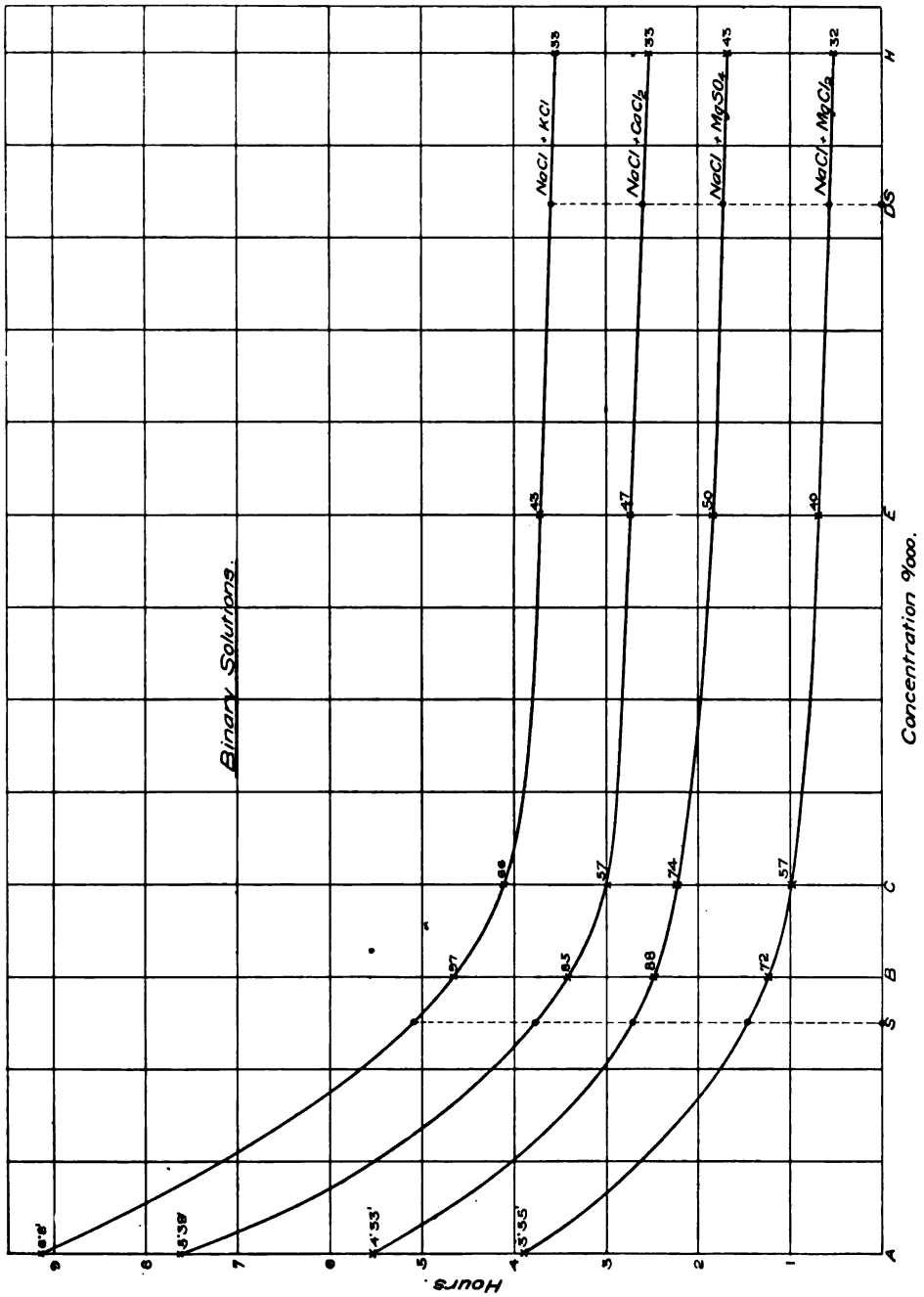

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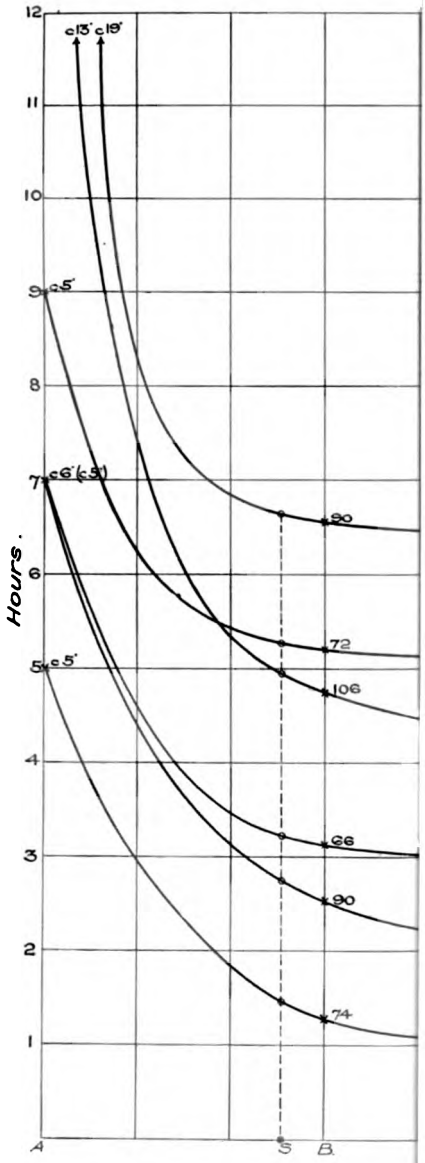

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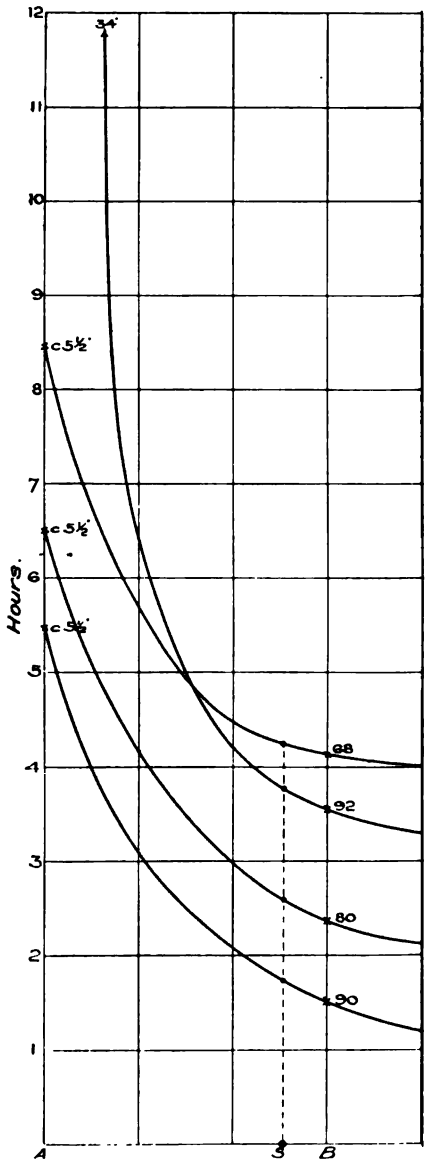

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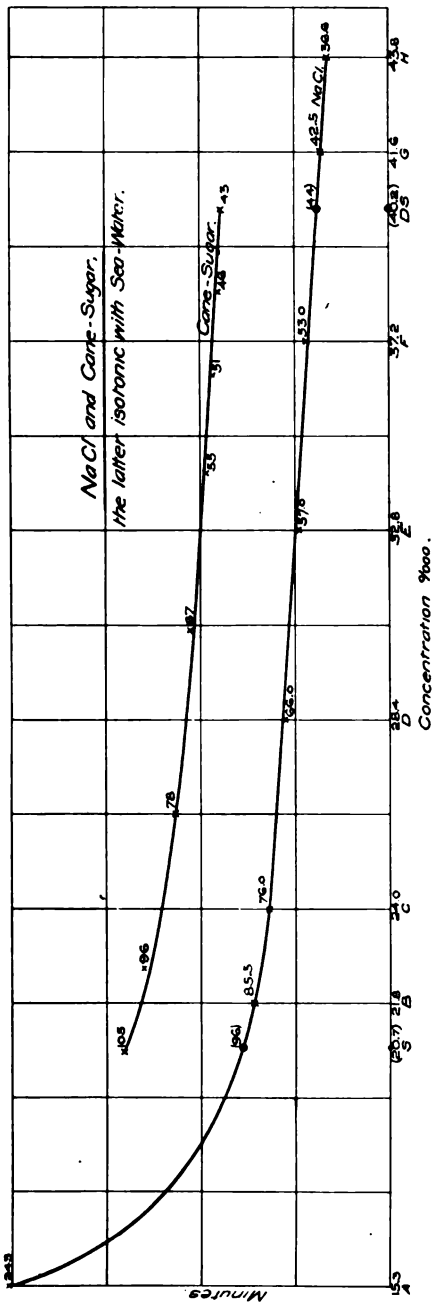

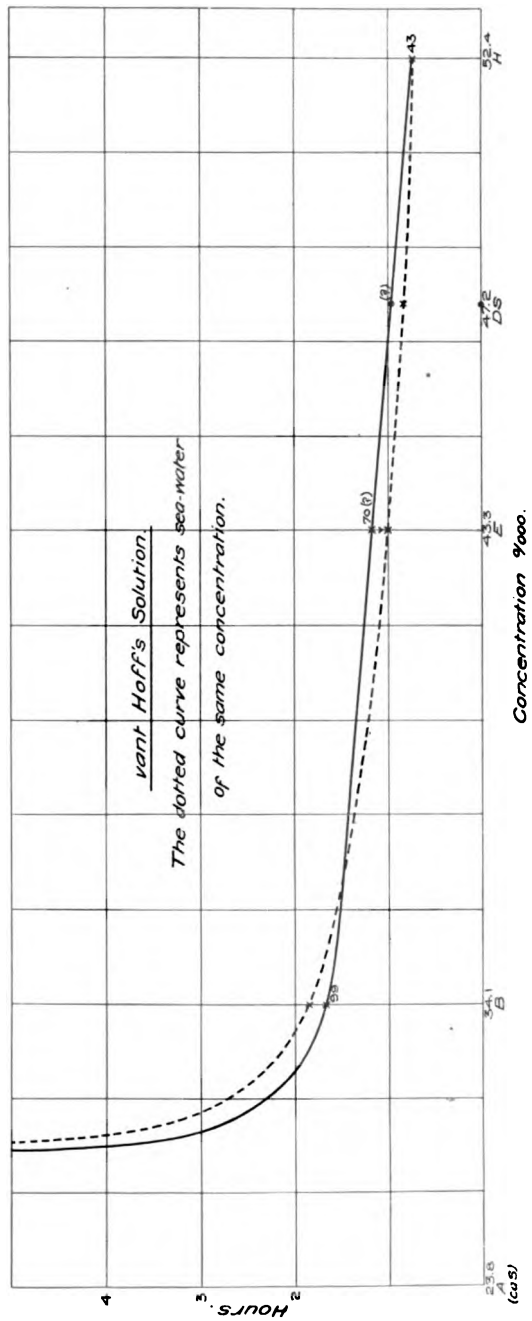

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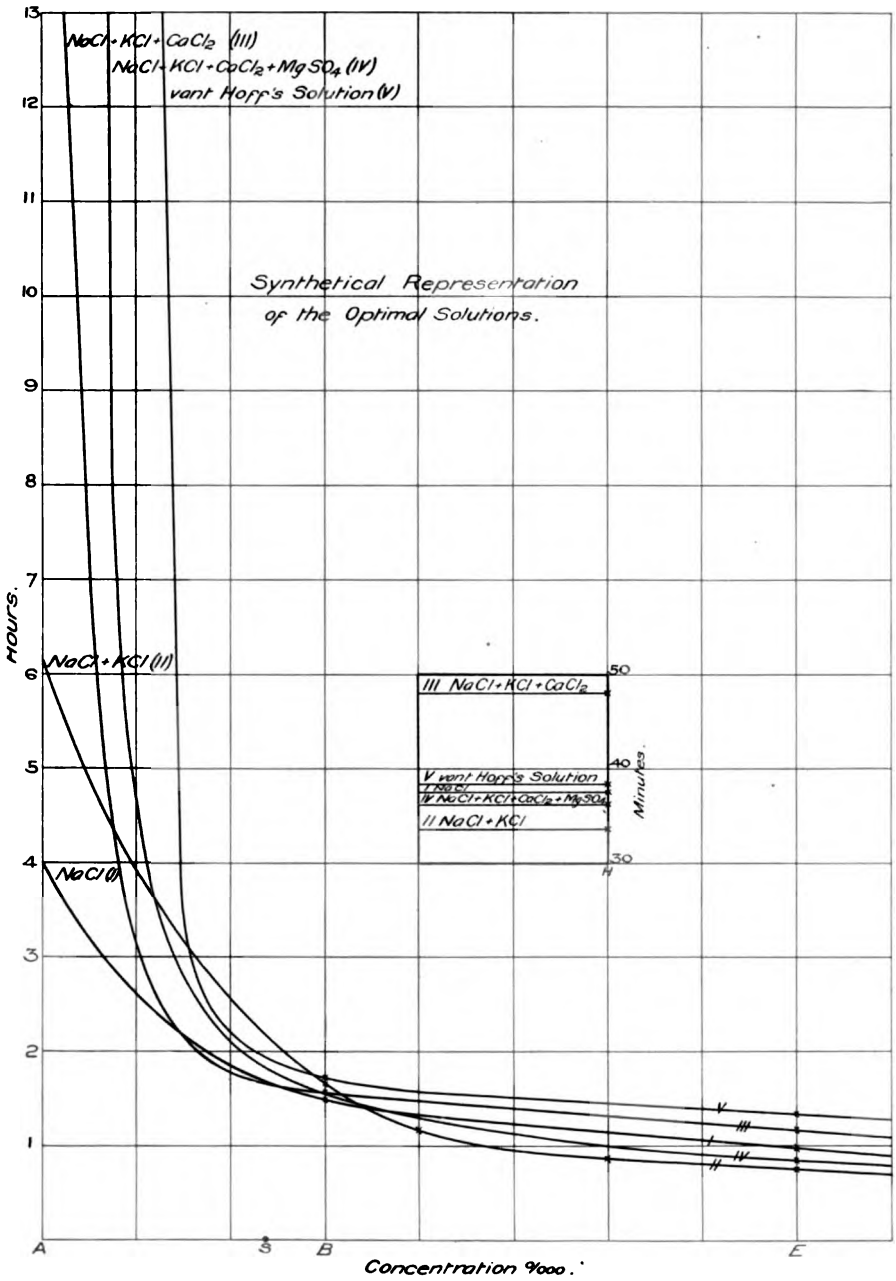

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UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 2, No. 19, pp. 193-215 November 24, 1905

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ON THE VALIDITY OF PFLÜGER'S LAW FOR THE GALVANOTROPIC REACTIONS OF PARAMECIUM[1]

BY

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For a long time it has been held by apparently all the investigators of the subject that the response of ciliate infusoria to an electrical current was essentially different from that seen in vertebrate muscle and nerve, and thus constitutes an important exception to Pflüger's law which was formulated for vertebrate material. In attempting to show that there is no ground for this belief but that really all the evidence available goes to show that the galvanotropic response of at least Paramecium to the galvanic current is in perfect accord with Pflüger's law, it will be advisable to show how the current conception originated.

I.—Historical Summary.

In the first place it must constantly be born in mind whenever organisms or their tissues are electrically stimulated, that they are affected in one way by a rapid change in the intensity of the current, such as is caused by making and breaking it, and in another way by the continuous flowing of the constant current. It is this first effect that is mainly responsible for the electrical stimulus of muscle and nerve, and the second effect that brings

[1] Translated from Pflüger's Archiv, Bd. 107, pp. 535-556.

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about galvanotropic, electrotonic, and electrolytic phenomena. (See Biedermann, 1895, pp. 166, 157–158; Loeb, 1897, pp. 439–440.)

In his Electrotonus Pflüger (1859) formulated, for nerves, the law that has since born his name. He showed that during the passage of the galvanic current there is a condition of increased excitability, catelectrotonus, in the region of the cathode; and at the anode a condition of diminished excitability, anelectrotonus. After the cessation of the current an opposite change of excitability persists for a short time. He furthermore showed that at the moment of the making of a constant current through a nerve the excitation is at the cathode and when the current is broken the excitation is at the anode. Subsequent investigation confirmed these results for muscles of various kinds, finding that here too the stimulus was at the cathode upon making, and at the anode upon breaking the current. (Biedermann, 1895, pp. 175–177.)

This stimulus was found to be transmitted along the muscle so that the whole length of the muscle contracted. So far the results for nerve and muscle are similar, but so far as the effects during the flow of the current are concerned there are differences. In the nerve the constant flow of the current produces only the condition of increased excitability but no actual excitation; while in the muscle a constant current of sufficient strength stimulates also during its flow and causes a contraction lasting as long as the current. (Biedermann, 1895, pp. 157–158.) This lasting contraction, however, is not propagated over the whole length of the muscle but is limited to the region of the cathode (Biedermann, 1895, p. 176). In view of these results on muscles the scope of Pflüger's law is ordinarily extended to include excitation in any tissue where it can be shown that the excitation occurs at the cathode upon making, at the anode upon breaking, or at the cathode during the flow of the current. It is in this sense that it will be used in this paper.

The earliest investigations of protozoa as regards their polar excitation by the galvanic current were by Kühne (1864, pp. 59–64), who found that in Actinophrys there was a progressive

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disintegration at the anode during the flow of the current. This he thought was not an electrolytic phenomenon, but he interpreted it as a tetanic contraction and thus arrived at a law of contraction for this animal essentially different from Pflüger's. Verworn (1889A, 1889B), confirmed and extended Kühne's results, finding anodal disintegration in many rhizopods and infusoria and in addition described the phenomenon of galvanotropism in protozoa. In all cases where both disintegration and galvanotropism could be demonstrated on the same animal he found anodal disintegration and locomotion towards the cathode. He interpreted the disintegration as a contraction or something equivalent due to anodal excitation (1889B, p. 294) and explains the galvanotropism of the infusoria as resulting from this excitation.

The anterior end he supposed to be most sensitive and thinks that the animal orients itself so that this end will be least stimulated. In some forms motion towards the anode was discovered, and Verworn supposed that in these cases the stimulus was at the cathode.

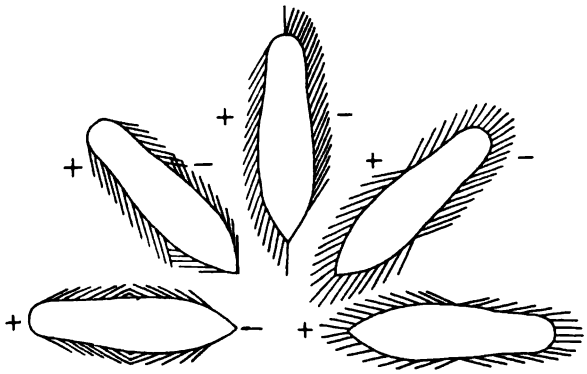

Fig. 1. The effect of the galvanic current on Paramecium in various positions. From *Ludloff, 1895*. The cathodal cilia point forwards, the anodal cilia point backwards.

Ludloff (1895) thoroughly analyzed the effect of the current on Paramecium, finding that the galvanotropic orientation was brought about by a polar effect on the cilia, the cilia on the anode side of the animal pointing backwards and beating more

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strongly backwards (fig. 1) while the cathodal cilia point forwards and beat more strongly forwards. Thus from a tranverse or oblique position the animal must be turned towards the cathode by both anodal and cathodal cilia. He found that the

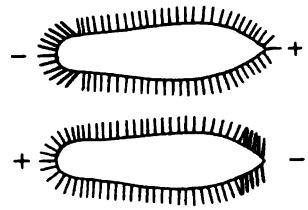

Fig. 2. Paramecium under the influence of a very weak current. From *Ludloff, 1895.* The cathodal cilia, which are the only ones that are influenced, are indicated by the heavier lines.

weakest effective currents influenced only a few cilia at the extreme cathode (fig. 2); stronger currents affected more of these cilia; and it was only with still stronger currents that the anodal cilia were influenced (pp. 540–541). In spite of these facts he maintained that the principal stimulation, which corresponds to the contraction of a muscle, occurred at the anode. Ciliary motion consists of a backward stroke (contraction) and recovery (expansion), he argued, and the current has a bipolar effect increasing the contraction at the anode and the expansion at the cathode. Therefore he thinks Pflüger's law does not hold here.

In a third paper Verworn (1896) upholds Ludloff's views and still further emphasizes his former contention that the contractile excitation is at the anode because it is there that the principal changes in the body of the Paramecium are observed. He adduces in this connection local anodal contractions reducing the posterior end to a narrow process (Zipfel) when it is at the anode and bending the whole animal into the shape of a crescent with the concavity towards the anode when a side is placed towards the anode. The throwing out of the tricocysts at the anode which was discovered by Ludloff is also brought forward as evidence for the anodal stimulation.

Loeb and Budgett (1897) point out the fact that it is not at all necessary to consider the exceptions to Pflüger's law in the

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same category as the phenomena that agree with it. They adduce evidence to show that the exceptions such as the anodal secretion of the skin glands of Amblystonia and the anodal phenomena in protozoa are produced by electrolytic changes in the fluid surrounding the organisms while the phenomena which agree with the law are due to electrolysis within the organism.

Subsequent authors have, in the main, shown the tendency to consider the disintegration phenomena and changes in the shape of the body apart from the galvanotropism of the infusoria and explain the latter solely by the action of the current on the cilia by means of which locomotion is affected. (Carlgren 1900, Pearl 1900, Kölsch 1902, Wallengren 1903A, 1903B.) I heartily agree with this view, and have mentioned the effect of the current on the body of the protozoan only to show how the current conception of the contractile stimulus at the anode and the expansive stimulus at the cathode originated. If Ludloff's paper had been the first one dealing with galvanotropism, it is very probable that it would have been universally recognized that, as has been pointed out by Jennings (1904, p. 489) the change which is responsible for the galvanotropism of Paramecium originates at and is mainly confined to the cathode.

II.—Criteria of Stimulation.

In this paper I will consider only the effect of the current on the cilia and not on the body, as it is evident that galvanotropism depends upon action of the cilia. Obviously, then, the first point to be determined is the criterion for stimulation. There might be some difference of opinion as to whether Paramecium in the unstimulated condition is swimming actively or at rest. As ordinarily examined Paramecium is swimming actively and comes to rest as the result of a weak contact stimulus. On the other hand in the cultures many animals are ordinarily at rest and begin to swim when disturbed in various ways. Leaving this question open, then, as hardly capable of a satisfactory solution it may be said that: *Paramecium in the unstimulated condition is either at rest or swimming actively forward.*

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Fortunately there is no such difficulty in determining the typical result of stimulation. The results of Jennings which

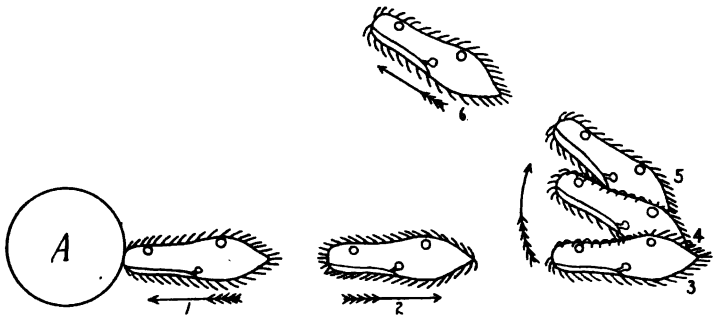

Fig. 3. Diagram of the motor reflex. From *Jennings*. *A*, Source of stimulus. 1-6, The successive stages of the motor reflex executed by a single Paramecium. The arrows indicate the direction of progression.

have been abundantly confirmed show us that Paramecium reacts to most stimuli (chemical, osmotic, heat, etc.) by (1), swimming backwards; (2), turning towards the aboral side; and (3), swimming forwards again (fig. 3). This series of changes has been called the motor reflex (Jennings, 1904, p. 450) and is

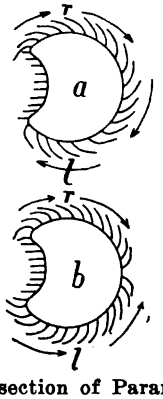

Fig. 4. Diagram of a cross-section of Paramecium, seen from the anterior end, and showing the oblique direction of the stroke of the cilia. From *Jennings, 1904*. *a*, Condition during the usual forward swimming: all of the body cilia strike obliquely backwards and to the right side; *b*, Condition during the turning towards the aboral side; the body cilia of the left side now strike obliquely backwards and towards the left. *l*, left side; *r*, right side. The arrows indicate the direction in which the cilia tend to move the animal.

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caused by (1), a reversal of all the cilia so that they beat forwards; (2), a return of the oral cilia to the backwards stroke, and a striking of some of the body cilia of the left side towards the oral groove (fig. 4), both turning the animal aborally; and (3), the resumption of the backward stroke by all the cilia. *The criterion of stimulation, then, is in general the execution of the motor reflex.*

III.—Galvanotropism and Polar Stimulation.

As has already been said in discussing Pflüger's law, galvanotropism is typically a phenomenon caused by the constant flow[1] of the galvanic current; and accordingly I will take up in this section only the phenomena observed during the flow of the current, leaving for subsequent consideration the stimulating effect of making and breaking the current.

The cardinal facts in galvanotropism are the pointing forward and the forward stroke of the cathodal cilia and the pointing backwards and backward stroke of the cilia at the anode end of the infusorian (Ludloff, 1895) (fig. 1). The current explanation of these facts is due to Ludloff (1895, p. 553), that the current exercises a bipolar effect, there being at the cathode an excitation of the expansion phase of the swing of the cilium, and at the anode an excitation of the contraction phase. To use Ludloff's words, "der galvanische Strom erzeugt an der Anode eine contractorische, an der Kathode eine expansorische Erregung." According to this view it is the anodal excitation to contraction, which would correspond with the contraction of a muscle, and therefore Pflüger's law does not hold. On the other hand Jennings (1904, p. 489), and apparently Statkewitsch,[2] hold that the action of the current is confined to the

[1] The fact that galvanotropism can be obtained with an interrupted current (Verworn 1889A, Birukoff 1899, Statkewitsch 1903) does not speak against this conception, for it is well known that if the alternations are rapid enough the interrupted current may act in the same way as the constant current.

[2] Statkewitsch's results on this subject are known to me only as discussed by Jennings.

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cathode.[1] Jennings (1904, p. 492) says in summing up the reaction to the electric current: "First the current stimulates in the same manner as any other stimulus; this stimulus has its origin at the anode. Second, the results of this stimulus are interfered with or overcome by an effect peculiar to the electric current, and having origin at the cathode. This peculiar effect is shown in a progressive reversal of the cilia, beginning with a weak current at the cathode tip, and gradually extending toward the anode end, until with a strong current it affects almost or quite the entire body."[2] *The view here maintained is that the stimulating effect of the constant flow of the current is in the main confined to the cathode, so that when the two poles are compared the anode must be considered the unstimulated and the cathode the stimulated pole. Therefore the response is in accordance with Pflüger's law.*

IV.—Evidence for the Condition at the anode.

All the investigators agree that the cilia at the cathode are stimulated during the flow of the current. What must be shown then to prove a unipolar stimulating effect of the current is that the *anode is in the unstimulated condition.*

The evidence may be grouped under four heads:

1. *Effect of weak currents on resting Paramecia:* Ludloff (1895, pp. 539–541) has shown that when the weakest currents are passed through *resting* Paramecia in gelatin solutions which enable the action of the cilia to be seen, only the extreme cathodal cilia are seen to contract rhythmically. No matter whether the anterior or posterior end or one side is cathodal, the same thing happens; the cathodal cilia contract and change their position so that they point more towards the anterior end of the animal (fig. 2). When the current stops the cilia come to rest again. All this time the anodal cilia have remained completely at rest.

[1] The position maintained in this paper had already been arrived at before the appearance of Jennings' paper, so that the evidence here adduced for the unstimulated condition of the anodal cilia is in the nature of an independent confirmation. It was in fact the attempt to explain the superposition of the chemical stimulation upon the galvanotropic reaction (to be described later) that led to the explanation of galvanotropism here set forth.

[2] Kölsch (1902, pp. 399-400) also criticises the idea of expansive and contractile stimuli, but does not say what the character of the stimulus is.

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When the strength of the current is increased slightly, the number of cathodal cilia that contract and point forward is increased; and the anodal cilia may be made to beat for a little while. They strike backwards, however, as they do when the animal is swimming in the unstimulated condition, and soon come to rest again.

When the current is still stronger, the cathodal area of reversed cilia is still farther increased and the anodal cilia continue to beat backwards during the flow of the current.

I have repeated these experiments of Ludloff for *resting* Paramecia, examining them in the viscid fluid obtained by soaking Irish moss (chondrus) in the Paramecium culture fluid for twenty-four hours as recommended by Statkewitsch (1904). This method cannot be too highly recommended. The fluid does not set like gelatin, is perfectly homogeneous, and absolutely inert, so that the Paramecia swim about and come to rest in a perfectly normal way. But they move so slowly that all ciliary

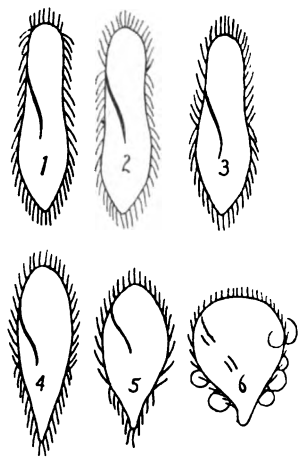

Fig. 5. Paramecium under the influence of currents of various strengths. From *Jennings, 1904,* after Statkewitsch. The cathode is above and the anode below. With a weak current only the extreme cathodal cilia reverse and point forwards, 1. As the current is increased (2, 3, 4, 5, 6) more and more cilia point forward until finally all the cilia point in that direction.

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motion obtained with weak currents can be easily followed.[1] My results confirm those of Ludloff in all points relating to the question here considered, and I think there can be no doubt that they prove that, *with respect to the cathode the anode is in the unstimulated condition.*

2. *Effect of the current on active Paramecia:* Jennings (1904, pp. 489–492) and apparently Statkewitsch[2] and the writer have confirmed the results of Ludloff mentioned above, but using Paramecia with cilia beating actively backwards. They find that the stronger the current the greater the cathodal region in which the cilia are reversed. Statkewitsch finds that as the currents get stronger this area of reversed cilia increases until it finally includes the whole animal (fig. 5).

I have devoted particular attention to the action of the anodal cilia in cases where they were beating backwards before the current was made and found that in some cases while the cathodal cilia promptly reversed upon making the current the anodal cilia continued beating exactly as before. In other cases, depending upon the strength of the current and the vigor of the animal, a slight increase in the rapidity of the contractions of the anodal cilia could be observed but they continued to beat backwards as they do in the unstimulated condition. Figure 6 illustrates this cathodal reversal in various positions of the animal.

3. *Chemical Stimulation during the flow of the current:* It might be maintained that in spite of the fact that the anodal cilia continue striking backwards during the flow of the current, they are not in an unstimulated condition as would appear at first sight: That just as the cathodal cilia are in a forced position, pointing forwards, so too the anodal cilia are in a forced position: That they can no more strike forwards than the

[1] The current used for the experiments described in this paper was obtained from the 110-volt power circuit of the University. It was led through a water rheostat, milliammeter, pole changer, key, and conducted to the preparation containing the Paramecia through Porter's non-polarisable boot-electrodes. When the cilia were to be examined microscopically, the animals were mounted on a slide in a thin layer of fluid, and the cover glass supported on strips of filter paper or Japanese lens paper with which the electrodes were connected by a drop of liquid.

[2] Cited from Jennings.

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cathodal cilia can strike backwards, but that this forced position is not so evident because it is the same that the cilia have in the normal unstimulated condition. If, however, the anodal cilia can be made to strike forwards during the flow of the current it will show that this objection is unfounded.

Loeb and Budgett (1897, p. 532) found that Paramecia when subjected to the current in a weak NaCl solution swam back-

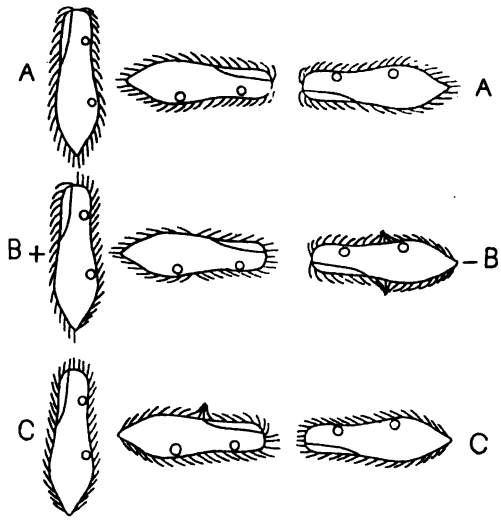

Fig. 6. Diagram of the polar effect of the galvanic current. *A A*, Before the closing of the current. *B B*, During the flow of the current. *C C*, After opening the current. After the closing of the current *B B* only the cilia of the cathodal half of the body reverse; after the opening it is, in general, only the cilia of the anodal half that reverse.

wards to the anode. Greeley (1903, 1904, pp. 25–27) finds that in acids Paramecia show a tendency to swim towards the anode, that alkalis inhibit this tendency, and that the same thing is in general brought about by solutions of many salts strong enough to act osmotically. Greeley makes no attempt to determine the effect of these various factors on the motion of the cilia. Normally they swim backwards to the anode, but sometimes they swim forward. Jennings (1904, p. 504–506) thinks that Greeley's results have no special significance for the theory

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of galvanotropism. He points out that the solutions cause the backward swimming without the current. When the current is made it orients the animals towards the cathode and so they swim backwards to the anode. Pütter (1900), Statkewitsch and Jennings (1904) have found in addition that after the animals have become acclimated to the chemical they swim as usual to the cathode.

I can confirm all of these results mentioned by Jennings. The swimming backwards to the anode has been found to take place in HCl, H_2SO_4, NaCl, and NaOH. If the Paramecia are immersed in one of these solutions of sufficient strength, they constantly execute the motor reaction, swimming backwards, turning, and going forwards again. If now the current is made, we have the galvanic response and the motor reaction is superimposed upon it. This comes out most clearly with a weak current and strength of solution which will stimulate but slightly, causing only some of the animals to execute the motor reaction. Under these conditions it may be seen that those animals which before the current was made were executing the motor reaction most vigorously, swim backwards to the anode after the current is made. Besides orienting the animals the current acts also as an additional stimulus, so that usually they now swim backwards for a longer distance than before the current was made. If, however, one animal be kept in view it can be seen that it swims rapidly backwards but soon slows down, stops, turns and swims forwards, becoming rapidly oriented towards the cathode again. Here we have all of the features of the motor reaction manifested in their usual way and there can be no doubt that we have the motor reaction, caused by the solution, superimposed upon the galvanotropic reaction.

If the current employed is stronger, the animal always remains oriented, even when it starts to go forwards after having gone backwards; but at this time the turning part of the motor reaction appears to be represented by its swimming in a very wide spiral. If the solutions used are stronger, all sorts of variations are introduced into the results which make them less clear. The turning phase of the motor reaction can no longer

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be detected, and sometimes the backward and forward phases can hardly be differentiated. The animals may move continuously backwards, the forward phase being represented by a slower backward motion with the anterior end describing wider circles.

Another way in which the modification of the galvanotropism by the reaction to chemicals was well shown is as follows: A trough 6 cm. long by 1.3 cm. wide was filled with the culture fluid in which the Paramecia had been living and a few drops of m/10 NaCl put in the center of it. Now the current was made and the Paramecia were introduced near the anode end. As they moved towards the cathode some of them came in contact with the diffusing NaCl and further progress ceased. They remained revolving rapidly on their long axes oriented towards the cathode and indicating by their aggregation the boundary

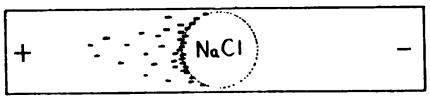

Fig. 7. Diagram of the aggregation of Paramecia under the simultaneous influence of galvanic and chemical stimulation. The Paramecia were introduced at the anode and swam towards the cathode until stopped by the presence of the Sodium Chloride solution (NaCl).

between the NaCl and the culture fluid as in fig. 7. As the NaCl continued to act, some of the animals were still more affected and moved rapidly backwards for several millimeters, then stopped and returned to the charge once more.

Let us return now to the bearing of all these phenomena on Pflüger's law. As was said in the opening paragraph of this section, the objection that the anodal cilia may still be in a forced condition incapable of a reversal will be met if it can be shown that the anodal cilia can be made to reverse during the flow of the current. *Now the backward swimming to the anode described above is brought about by a reversal of the anodal cilia.* This was determined by a microscopic observation of the cilia in the jelly from quince seeds recommended by Statkewitsch (1904). This jelly was made up with an m/10

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solution of NaCl, the Paramecia mixed with it, and the whole covered by a coverglass. After considerable searching it was then possible to find Paramecia which were moving both backwards and forwards and whose cilia could be observed. It was thus seen that as they moved backwards towards the anode, all of the cilia were striking forwards, and as they moved ahead again towards the cathode the cilia on the anodal half of the animal reversed and beat backwards. I think this shows conclusively *that during the flow of the current the anodal cilia are in the unstimulated condition, and not in any forced condition; for they are still capable of responding to chemical stimulation by the usual reversal.* The cathodal cilia on the other hand always remain reversed when the currents are strong enough, though the variation in the width of the spiral observed in chemical stimulation is probably to be interpreted as a striking of the left hand cilia obliquely forwards and *towards* the oral groove (fig. 4) instead of away from it as usual (Jennings, 1904, p. 453).

One fact which at first sight would seem to furnish evidence against the view here maintained must be mentioned. When a Paramecium is moving backwards and all its cilia are striking forwards, the making of the current causes a reversal of the *anodal* cilia which assume the unstimulated condition, striking

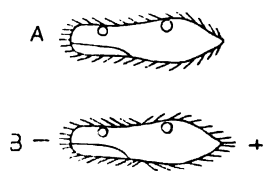

Fig. 8. Diagram of the action of the current on Paramecia that are already swimming backwards. *A*, Before closing; the cilia strike forwards and the animal swims backwards. *B*, After the closing; the anodal cilia reverse and point backwards.

backwards (fig. 8). This can best be shown by breaking the current after the animal has become oriented. The infusorian immediately swings backwards and if the current is strong enough the reversed stroke of the cilia lasts for quite a while. But the

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instant the current is made again the anodal cilia strike backwards, and continue doing so as long as the current lasts. This shows without doubt that the current does affect the anodal cilia but it does not show that it *stimulates* them. It indicates on the other hand, that it tends to inhibit the stimulated condition, and this is just what we should expect if Pflüger's law holds. For, according to this law, there is at the anode a condition of lowered excitability, and in the case in point, in this lowered condition the previous stimulus is no longer capable of keeping the anodal cilia in the stimulated reversed condition. But the fact that these cilia are still capable of responding to chemical stimuli shows that they are in an unstimulated and not a forced position.

4. *Effect of strong currents:* Many observers have described a backward swimming towards the anode when very strong currents are employed; and Statkewitsch has shown that under these circumstances the cilia all over the body strike forwards. This might be interpreted as a lack of any polar effect on the cilia by very strong currents. I think, however, that it should not be so interpreted. The animal is strongly contracted and is evidently injured and it is this secondary injurious effect that furnishes the stimulus resulting in the reversal of the anodal cilia.

V.—Effects of Making and Breaking the Current.

As already indicated, making and breaking the current, that is, rapid variations in intensity, characteristically produce effects that start at one pole but are conducted from that pole all over the animal or tissue in question. In muscles and nerves this stimulus is at the cathode upon making the current and at the anode upon breaking it. The only investigators who have specially studied this point for Paramecium are Roesele (1902) and Statkewitsch (1903, p. 47). They both used induced currents and came to the conclusion that the stimulus was at the anode and that hence Pflüger's law does not hold. Now it must be acknowledged, I think, that safe conclusions about the validity of this law cannot be reached from experiments with induced currents,

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for in these cases we may have stimulation produced by (1) making the current, (2) the flow of the current, (3) breaking the current, and as the current is instantaneous there is no way of separating these effects. Now while it has been found for vertebrate tissues that weak induced currents stimulate mainly at the cathode, and hence upon making, in some forms breaking the galvanic current is a more effective stimulus than making it (for instance *anodonta*, Biedermann, 1895, p. 161), and we should expect in these cases that a weak induced current would stimulate upon breaking. For these reasons I have investigated the effect of breaking and making the galvanic current upon the cilia of Paramecium.

Effect of Making the Current: The weakest currents, as Ludloff has shown and as I can confirm, for resting Paramecia have an effect only at the cathode. When the current is made so that the anterior end is cathodal (homodrom current) this current makes the extreme anterior cilia, which were formerly quiescent, beat in indefinite directions, but mainly backwards. Slightly stronger currents I found make them beat towards the oral side and still stronger ones make them strike forwards. No change whatever could be seen except at the cathodal tip. When the current was broken there was no stimulus and the cilia resumed their quiescent condition.

When the posterior end was cathodal (heterodrom current) similar results were obtained. But with the same strength of current the posterior end was affected to a greater extent, probably because as in Loeb's experiments on the secretions of the skin glands of Amblystoma (Loeb and Budgett, 1897, p. 424), the tapering of the pointed posterior end presents a greater surface which the current lines cut as they leave the body. The weakest currents made the cilia strike indefinitely. Stronger ones made them strike forwards.

Medium and strong currents upon being made merely brought about the characteristic reversal of the cilia on the cathode half of the animal and increased or initiated the backward stroke of the cilia on the anodal half. In no case when the animals were examined in their normal culture fluid or indifferent media

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could a backward jumping of the animal and a reversal of the anodal cilia be observed.

In all these cases it will be noticed that the making of the current only brings about the condition which exists during the flow of the current. The stimulated condition is limited to the cathode and does not appear to spread to the rest of the animal. There are, however, some facts which indicate that the making of the current produces a general stimulation extending to all parts of the animal. These are, first the results of Pearl (1900) and Jennings (1904) showing that the orientation to the current is brought about by a combination of what Pearl calls the forced movement factor represented by the characteristic galvanotropic positions of the cilia and the reflex factor which includes some of the features of the motor reflex. The reflex factor evidently depends upon a stimulation of the whole animal.

Secondly, in Paramecia which had been gradually acclimated to m/18 NaCl I found, as Pütter (1900, p. 297) and Jennings (1904, p. 505) had found, that the animals were no longer stimulated by the chemical, that they were not performing the motor reaction when immersed in it, and consequently swam forwards to the cathode when the current was made. But I also found that in this case the animals did not move forward immediately after the current was made, but either swam backwards or remained stationary for a while, turning on their long axis, and only later swam forwards to the cathode. This shows that we have here, just as in the case of the chemical and galvanic stimuli, motor reaction superimposed upon the usual galvanotropic reaction. And this motor reaction probably has been produced by conduction of the make stimulation over the whole animal. This different behavior of the Paramecia when acclimated to NaCl is a question of considerable general significance and I hope to carry the matter farther at a future time, for it may be possible by means of this method to gain some insight into the part played by the ions in stimulation.

Effect of Breaking the Current: As has already been said, the breaking of the weakest currents has no visible stimulating effect; the cathodal cilia merely return to their unstimulated

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condition and the anodal cilia are not affected. As the currents are made stronger, many observers have noted the quick jerk backwards that follows the breaking of the current. An examination of the ciliary movements in viscid media has shown that as was to be expected this is accomplished by a forward stroke of all the cilia. Thus when a homodromal current of sufficient intensity is broken, the posterior anodal cilia immediately reverse while most of the anterior cathodal cilia remain reversed as they were during the flow of the current. The cilia of the region of the oral groove, however, immediately strike backwards as the current is broken, so that as the infusorian is carried backwards its anterior end swerves towards the aboral side (fig. 6). With weaker currents it frequently happens that this swerving occurs without any backward motion.

When a strong heterodrom current is broken, the posterior cathodal cilia remain reversed as they were during the flow of the current, while all of the anodal cilia also become reversed so that the animal jerks back (fig. 6). It is to be noted, however, that the strength of current required to bring about a reversal of the anodal cilia is considerably greater when the anterior end was the anode than when the posterior end was the anode before breaking. This difference is probably due to the broad tapering shape of the posterior end by means of which the surface of the physiological anode is increased. It may, however, be due to a greater sensitiveness of the posterior end.

Summing up the effects of making and breaking the current it is evident that at making the only place where ordinarily the cilia pass from the unstimulated to the stimulated condition is at the cathode; and at breaking the only place where the cilia pass from the unstimulated to the stimulated condition is at the anode. These results agree strictly with Pflüger's law.

VI.—Comparison with the Effect of Induced Currents.

My results have a bearing on the interpretation of the effect of induced currents in three respects.

1. *Is the make or break the more effective stimulus?* This

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question has been answered both ways in my experiments. Sometimes the weakest currents stimulated only at making, and sometimes only at breaking; and it appeared that the Paramecia were in the most normal condition when the make was the most effective stimulus.

2. *The response to an induction shock* is ordinarily a motor reaction (Statkewitsch, 1903), and this is under ordinary circumstances obtained by breaking and not by making the galvanic current. *It would seem then that the usual response to the induced current is the result of the break stimulus; and this, of course, should be at the anode according to Pflüger's law.*

3. *The origin of the wave of reversal of the cilia* at the anode appears to be the principal evidence depended upon by Statkewitsch to disprove the applicability of Pflüger's law to ciliary stimulation. As the cilia reverse, they move so rapidly that in most cases I have been unable to see that the reversal began sooner in one place than in another. In those Paramecia, however, in which the break proved a more effective stimulus than the make I could see that when a homodromal current was broken there was a latent period of about one second before the reversal took place and when a point in the anterior third of the animal was watched it could be seen that the cilia posterior to that point reversed first and the wave of reversal spread rapidly anteriorly. This looked like an anodal origin for the wave of reversal; but when a heterodromal current was tested it was found that in this case the wave of reversal also traveled from behind forwards. My results would seem to indicate then that the *direction of the wave of reversal depends upon the structure of the animal.* I do not wish to imply, however, that the results of Statkewitsch are incorrect, for it is easily possible in experiments performed under such different conditions that different responses should be obtained, especially when they deal with such a variable response as the ciliary wave. Thus for instance, when Paramecium is swimming normally forwards in its culture fluid which has been cooled down so that the ciliary waves can be seen they progress anteriorly, but when the infusiorian is

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swimming forwards through viscid media the waves move backwards (fig. 9).

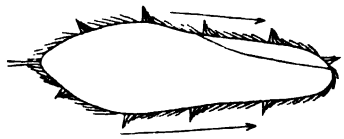

Fig. 9. Diagram of the ciliary waves while the Paramecium is swimming quietly forwards in the cooled culture fluid. Both the animal and the waves move forwards as indicated by the arrows.

It is evident, then, that when interpreted in the light of the results obtained with the galvanic current the effects produced by induced currents do not justify the conclusion that Pflüger's law does not hold for the stimulation of the cilia in Paramecium.

VII.—Characteristic Action of Electric Current.

In the light of these results then it is evident that the characteristic action of the galvanic current on the cilia of Paramecium is that while an ordinary stimulus, whether localized or unlocalized, affects the whole of the animal, bringing about the motor reaction, the effects of the stimulus produced by the make and flow of the current are not usually distributed over the whole of the animal, but remain confined to the region of the cathode. Here as elsewhere, then, the peculiarity of the electric current is its polar action; and since the pole at which the principal changes occur is the cathode, it is to the cations that we must look for the futher elucidation of the problem.

These results have been worked out for Paramecium only, but in view of the fact that Pearl (1900), Kölsch (1902), Wallengren (1903A, and 1903B), and others have shown that so many other infusoria have the same characteristic reversal of the cathodal cilia; and since Wallengren has shown that cathodal, anodal, and transverse galvanotropism are produced by the same kind of electrical excitation, it is probable that Pflüger's law holds for the excitation of the cilia of all ciliate infusoria.

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VIII.—Summary.

1. The cilia in the unstimulated Paramecium are either at rest or striking backwards.

2. When stimulated, Paramecium usually executes a series of movements the principal feature of which is the reversal of the cilia so that they now strike forwards.

3. Adopting the reversal of the cilia as the criterion for stimulation, Pflüger's law holds for the stimulation of the cilia. For upon making and during the flow of the current the cathodal and only the cathodal cilia reverse, and upon breaking the current the anodal cilia reverse.

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BY

C. W. WOLFGANG OSTWALD

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